1 **Exploiting flow-based separation techniques for sample handling in wine analysis**

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7 **Abstract**

8 Wine is a fermented product consumed in a large scale all over the world, therefore has a large impact

9 both economic and food safety terms. The analytical control of the final product is thus of high

10 importance; it is not a simple task given that the chemical composition of wine is very variable and

11 complex. Consequently, there is always the need for some sample pre-treatment prior to analysis.

12 Flow-based analysis are known for their efficiency in sample manipulation, and can be easily coupled

13 to other techniques, such as separation techniques, namely membrane-based or extraction procedures.

14 This possibility is an important step when dealing with complex matrices, such as wine samples.

15 This review presents the state of the art of the methodologies that were developed using flow-based

16 systems coupled to separation devices applied to wine analysis, namely membrane-based, solid, and

17 liquid phase extraction and low pressure chromatography separations.

18 **Keywords:** flow injection analysis; sequential injection analysis; lab-on-valve system; membrane-

19 based separations; solid and liquid phase separations; wine analysis

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1. Introduction

Wine is an alcoholic beverage produced from the fermentation of grapes. It is produced since ancient times, in fact there are archaeological records of wine that goes back to more than 7.5 thousand years including some unequivocal evidence of winemaking from a region in Egypt some 5 thousand years ago (Jackson 2008). Since then, wine has been a product consumed in a large scale all over the world that has a huge impact regarding both economic and food safety terms. The analysis is thus of extreme importance to guarantee the quality and safety of the final product. This task is challenging due to the wide variety of wines that are produced; different compounds can be present, at different concentrations ranges (Segundo et al. 2004), being the majority of the chemicals found in wine derived from metabolic by-products from yeast activity (Jackson 2008).

Wine is mainly composed of ethanol, sugars, organic acids, polyphenols, and proteins, as well as several inorganic species including heavy metals (Galani-Nikolakaki et al. 2002; Pyrzyńska 2004; Pohl 2007; Jackson 2008). Besides the complex matrix, wine colour can be another challenge particularly regarding spectrophotometric reactions. Therefore, the analytical procedure has to be carefully considered to allow the selective determination of the multiple analytes and overcome potential interferences from colour and other compounds that are not of interest and may be present in higher abundance. To avoid these interferences and improve the sensitivity, accuracy, and reproducibility of the methods, there is always the need for sample treatment prior to analysis in the form of dilution, solid phase extraction or liquid-liquid extraction (Tóth et al. 2008). Sample pre-treatment is usually laborious, time consuming, and requires skilled personnel. Ideally, these steps should be carried out in-line with the detection to provide real-time measurements. Additionally, automation of these analytical steps by the use for flow-based methods through miniaturization and automation presents several advantages such as improved throughput and decreased consumption of reagents and samples. Flow injection analysis (FIA) was first introduced by Ruzicka and Hansen in 1975 (Ṙužička and Hansen 1975) and consists in a continuous steam of reagent where a precise volume of sample is introduced and propelled towards the detector. Later, Ruzicka and Marshall (Ruzicka and Marshall 1990) presented a new approach named sequential

injection analysis (SIA) where the flow system makes use of a programmable flow, not continuous anymore; where a precise volume of reagent and sample are aspirated sequentially into a holding coil and propelled towards the detector by reversing the flow. Further miniaturization of this approach can be used with the so-called lab-on-valve (LOV) system (Ruzicka 2000) that makes use of the programmable flow approach at a microliter scale instead of the millilitre scale used in SIA.

Flow-based methods have proved to be an efficient tool for wine analysis. In fact, some reviews have been published reporting the importance of the flow-based systems in routine determinations in wines (Segundo et al. 2004) and in the quality control of wines and in oenological laboratories (De Castro et al. 2003; De Castro and González-Rodríguez, J.Pérez-Juan 2005). Most of the reference methods (Compendium of international methods of wine and must and Analysis-OIV 2014) have not been changed for a long time, and the flow-based systems, continuous methods, present good precision for winery necessities making them suitable not only to evaluate the quality of the final product but also the monitor the fermentation and post-fermentation process.

Most of the published works couple separation techniques to flow-based systems in order to overcome all the challenges mentioned previously. Figure 1 represents the cumulative evolution of the published works up to 2020 that make use of separation techniques coupled to flow-based systems for wine analysis.
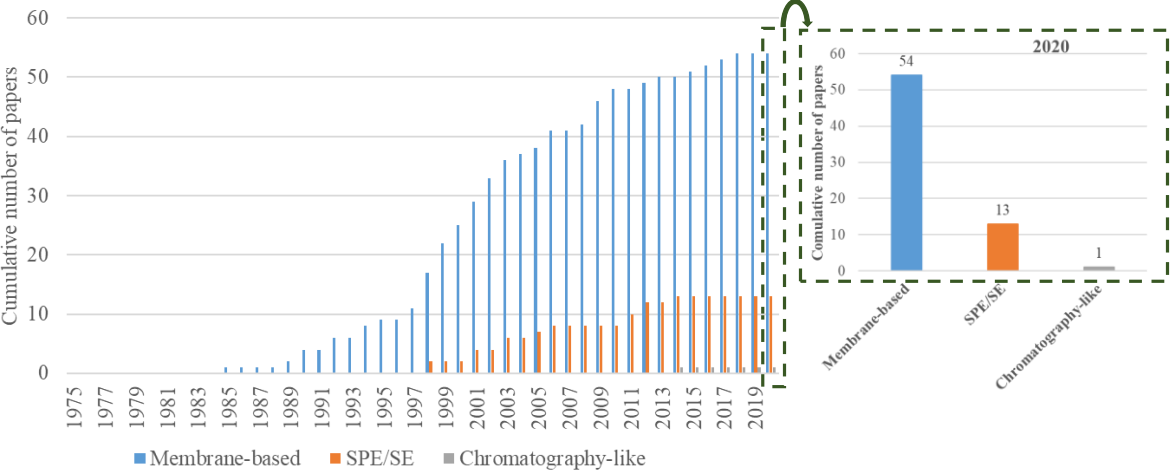
Figure 1 Evolution of the scientific literature published dealing with separation techniques coupled to flow-based systems applied to wine analysis up to 2020.

The use of separation techniques coupled to flow-based systems applied to wine analysis has been reported since the mid-eighties. The membrane-based separation techniques were the first to be used, and, from 1997 to 2009, there was an exponential increase on the published papers that reached a plateau afterwards. The use of separation techniques by solid or liquid separation are not so common for this type of analysis; in Figure 2 the percentage distribution of the works by type of separation technique used is represented.
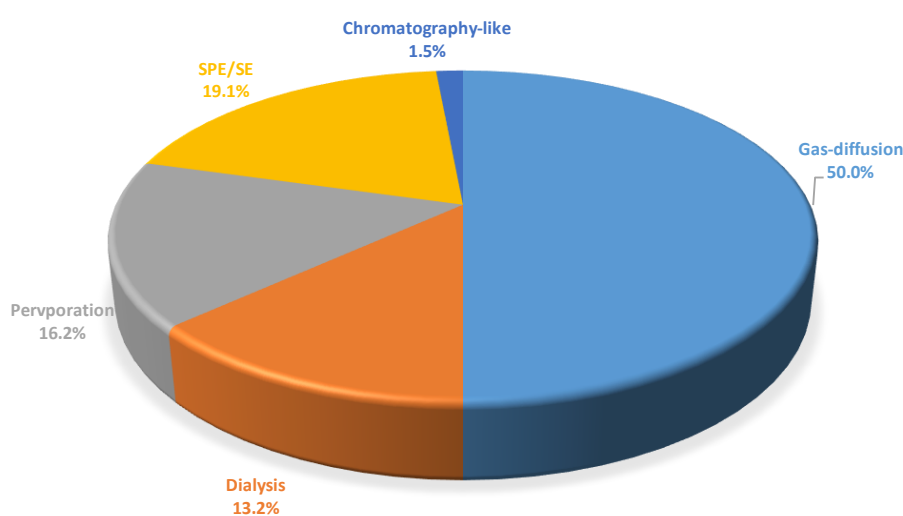
Figure 2 Percentage of number of papers published up to 2020, dealing with different separation process applied to wine analysis.

This review presents the state of the art of the use of separation processes coupled to flow-based systems applied to wine analysis, namely membrane-based, solid, and liquid phase extraction and low pressure chromatography separations.

2. Separation techniques coupled to flow-based systems applied for wine analysis

2.1. Membrane-based separations

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1 The use of a separation procedure by means of a membrane-based technique may provide the

2 elimination/reduction of interferences but also an efficient in-line dilution. The efficiency of the

3 procedure depends on nature of the membrane, type of surface, porosity, thickness, the path length, and

4 geometry (Vidigal and Rangel 2015). The nature of the membrane, its hydrophobicity, will make the

5 characterization of the separation technique in terms of gas-diffusion, or dialysis. When a hydrophobic

6 membrane is used, depending on the main configuration of the unit of separation, there can be gas-

7 diffusion or pervaporation; if the membrane is hydrophilic, we are in the presence of a dialysis

8 technique. Different configurations of the units supporting the membrane can be used, and will define

9 the path length and geometry. The units used for the gas-diffusion and dialysis methods have the same

10 configuration and the main difference from the units used in the pervaporation methods is the existence

11 or nonexistence of a spacer between the membrane and the donor stream. In the gas-diffusion and

12 dialysis units, the membrane is in contact with both streams; in the pervaporation unit, there is a spacer

13 between the donor stream and the membrane, which will make a significant difference in terms of

14 efficiency of the mass transfer unit (Luque de Castro 2008).

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16 *2.1.1 Gas-diffusion*

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18 Gas-diffusion is generally used to separate volatile compounds from a matrix by a concentration

19 gradient using a hydrophobic membrane (Santos et al. 2020). Gas-diffusion units (GDU) are usually

20 coupled to flow-based systems with the intent of eliminating or reducing the interference of some

21 sample matrices. Most of the published papers reporting the use of this technique for wine analysis

22 have, as principal strategy, the separation of the volatile fraction from the sample matrix. This technique

23 was used for the determination of the content of ethanol, carbon dioxide, free or total sulphur dioxide,

24 acetic acid, volatile acidity, among others, in wine and related samples (Table 1). As it can be noticed,

25 all of these compounds can be easily separated from the aqueous phase due to their volatility.

26 Most of the developed methods make use of flow injection manifolds, instead of sequential injection

27 systems. Since there is a continuous flow, in flow injection systems the membrane is always

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conditioned, ready to use. In sequential injection, since the flow is programmable, there is the need for an additional step to condition the membrane, thus this methodology can be more time consuming. Without the conditioning step, the efficiency of the mass transfer through the membrane can be reduced. Alternatively, an additional peristaltic pump can be coupled to the system connecting one of the streams of the separation unit, usually the acceptor stream (Segundo and Rangel 2001; Chinvongamorn et al. 2008), being this stream in constant flow, which makes the membrane always ready to use. The methods that make use of this approach (Segundo and Rangel 2001; Chinvongamorn et al. 2008) presented lower values of relative standard deviation (RSD), when compared with works without the additional driving device (Pais et al. 2013; Vidigal and Rangel 2017) (Table 1).

The developed methods were mostly used in the determination of sulphite and ethanol, since these two compounds are volatile, or converted to volatile as it is the case of sulphites. Carbon dioxide, urea, acetic acid and ascorbic acid, were also determined with the presented works.

2.1.2. Dialysis

Dialysis systems are often used to perform an in-line dilution and/or to eliminate matrix interferences like high molecular weight species and ions. This diffusion, that makes use of a hydrophilic membrane, can occur due to a concentration gradient or due to an ionic strength gradient through the membrane; if the membrane has an inert or active role on the diffusion, respectively (Santos et al. 2020). In this technique, the sample is propelled towards the donor channel of the diffusion unit, and only a portion of the sample will pass through it for the acceptor steam, prior or after reaction, towards detection. In order to enhance the efficiency of the transfer through the membrane, Mataix and Luque de Castro (Mataix and Luque De Castro 2001), presented an approach where the acceptor channel of the dialysis is placed in the loop of an injection valve, thus increasing the time of contact between the samples and the acceptor stream. As this stream is stopped while the sample passes through the donor stream, a concentration of the reaction product is attained.

1   A summary of the published works dealing with flow-based systems coupled to dialysis separation

2   techniques for wine analysis is presented in Table 2; most of these works employ photometric detection

3   systems. Therefore, besides accomplishing sample dilution, it is possible to reduce most of the

4   interferences derived from the coloured compounds usually present in the sample, as it is the case of

5   the coloured wines, like rose and red wine. These separation procedures are very efficient in the

6   performance of the clean-up of the sample.

7   This approach was used on the determination of reducing sugars, organic acids and titratable acidity.

8   Unlike what happens in gas-diffusion separation methods, here the separation technique is applied for

9   non-volatile compounds.

10   A dialysis membrane can also be used to entrap electrodes and sensors on determinations that make use

11   of electrochemical detection systems (Matsumoto et al. 1989; Groom et al. 1993; Lobo et al. 1996;

12   Campuzano et al. 2007; Vargas et al. 2016). This approach is used to improve selectively and to increase

13   the lifetime of the electrodes, since the membrane can prevent aqueous soluble species from dissolving

14   out (Lobo et al. 1996); and when applied to biosensors, the enzyme or the bioanalyte have higher

15   durability (Vargas et al. 2016).

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17   *2.1.3. Pervaporation*

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19   Pervaporation (Luque De Castro and Papaefstathiou 1998; De Castro et al. 2003; Luque de Castro 2008)

20   has been described as a separation technique where the volatile fraction goes from the donor channel

21   towards the acceptor channel, transferring through an air gap and a porous membrane. It is a

22   combination of two separation principles, evaporation and gas-diffusion. The headspace between the

23   donor channel and the membrane is claimed to be an advantage when compared to simple gas-diffusion

24   units since the sample is not in contact with the membrane, thus preventing clogging and damage.

25   Therefore, it could be possible to manipulate samples with more complex matrices, like suspensions.

26   In 1990 (Prinzing et al. 1990), this approach was firstly used coupled to a flow injection analysis system

1   to monitor a fermentation procedure. Later, Luque de Castro (Mataix and Luque De Castro 1998) used

2   the same approach for the determination of total and free sulphur dioxide in red and white wines.

3   Similar to pervaporation, a "membraneless gas-diffusion (MGD) unit" was also developed

4   (Choengchan et al. 2006), where the donor and the acceptor channels are not separated by a membrane

5   but are parallel to each other and separated by a thin wall (Ratanawimarnwong et al. 2013). The

6   separation by MGU consist in the diffusion of the volatile analyte from the donor channel for the

7   acceptor channel thought the headspace. This will imply a change on the physicochemical properties of

8   the acceptor stream that can be detected (Alahmad et al. 2018).

9   A summary of the application of pervaporation coupled to flow-based systems for wine analysis up to

10   2020 is resumed in

Table 3. It was possible to observe that only flow injection systems were used on the development of the proposed methodologies. And like in gas-diffusion separations, the methodologies with pervaporation separation were used volatile compounds. On the other hand, this technique showed to be less repeatable than gas-diffusion since higher values of RSD were observed when the application for the same analyte is compared (i.e.: ethanol determination by gas-diffusion vs ethanol determination by pervaporation; and sulphite determination by gas-diffusion vs sulphite determination by pervaporation).

2.2. Extraction techniques

In extraction techniques, as the name implies, it is possible to isolate the analyte of interest, or some contaminant, by removing it from the matrix. To do this, the sample that contains the analyte has to be merged with another phase, liquid or solid, to which the analyte will migrate, separating it from the original matrix. Liquid phase extraction, usually named as liquid-liquid extraction (LLE) or solvent extraction (SE), indicates that the phase that will extract the analyte has to be a liquid one; and the solid-liquid extraction is usually named as solid phase extraction (SPE), where the analyte of interest will be retained in a solid sorbent. The extraction using solid or liquid phases prior to analysis are useful techniques where it is possible to attain some elimination of interferences due to the extraction of the analyte of interest form the original matrix and at the same time the pre-concentration of the analyte is achieved. In order to fulfil the requirements of a greener analytical chemistry, it is possible to observe the development of several methods involving the use of reduced volumes (order of microliters) of solvents (Kocúrová et al. 2013). Considering the principles of Green Chemistry, one of the major disadvantages pointed out for conventional extraction techniques is the use of large volumes of solvents or sorbents, in order to attain an efficient extraction. The possibility to perform this technique in a flow-based system allows the minimization of the consumables amount to be used.

2.2.1. Solid phase extraction

Solid phase extraction is usually one of the chosen techniques to carry out the sample pre-treatment that is also easily coupled to flow-based systems. Therefore, the extraction is carried out in an automatic way with significant reduction on the amount of the reagents used with high sample throughput (Motomizu and Sakai 2008).

In this technique, the sample flows through the solid sorbent, where the analyte of interest is retained, and later eluted towards the detector. After elution, the solid particles can be regenerated in order to perform the next cycle, to reuse the column and perform several analysis which reduces the cost of analysis. One limitation of reusing sorbents is that these might become saturated or too packed over time, increasing backpressure (Vidigal et al. 2013). In order to overcome these disadvantages, a reusable approach can be used, where a new column is built every assay. By using the LOV platform, it is possible to easily pack the particles into the flow cell in a miniaturized size. Therefore, no contamination between consecutive assays is guaranteed, as there is a new column for each cycle. Additionally, as the analyte is retained in the surface of the solid support in the flow cell, solid phase spectrometry (SPS) can be performed. In SPS, the quantification of the analyte is carried out by measuring the light attenuation directly in the solid support, and not in the eluate. SPE comprises two steps: fist the analyte is retained in the solid support, then the analyte is eluted towards detection. On the other hand, SPS consists of only one step, retention and detection in the surface of the solid support. When the analyte is retained in the solid support, its pre-concentration is attained. With the elution towards detection there may be some loss of this preconcentration. By performing SPS instead, no analyte loss pre-concentration can take place, as there is no elution step. (Vidigal et al. 2011). LOV is the ideal platform as it allows the manipulation of beads as well as incorporation of the detector in the same unit.

As presented in Table 4, only two of the reported works make use of a renewable column to perform SPS, where a new column of nitrilotriacetic acid (NTA) resin was formed in the flow cell of the LOV platform at the beginning of each analytical cycle. These NTA resins chelate metal ions and thus they were used in the quantification of iron (Vidigal et al. 2011) and proteins (Vidigal et al. 2012). On the

1   other hand, a reusable approach is frequently used with the conventional C18 sorbent for the

2   determination of metals, mainly lead (Bakircioglu et al. 2003, 2011; Pires Fernandes et al. 2003; Wan

3   et al. 2006) but also for copper (Pires Fernandes et al. 2003) and cadmium (Pires Fernandes et al. 2003).

4   Others used this approach for the determination of organic particles such as polyphenols (Arce et al.

5   1998b; Wang et al. 2012), anthocyanin's (Mataix and Luque de Castro 2001), and biogenic amines

6   (Arce et al. 1998a).

7   Other types of sorbents like biological materials, such as bacteria, can also be used in the separation

8   procedure, the so-called biosorption (Bakircioglu et al. 2011). These biomaterials are used due their

9   large available quantities, good performance, selective adsorption, low cost, free availability and

10   regeneration. At the same time these can be used in a wide range of conditions, such as pH and

11   temperature, presenting high biosorption capacity due to the functional groups present in the bacteria

12   cell wall (Bakircioglu et al. 2011).

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15   *2.2.2. Liquid-liquid extraction*

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17   In liquid-liquid extraction (LLE), the extraction occurs between two immiscible liquids, usually an

18   aqueous phase, the sample, and an organic one, extraction phase. The major disadvantage of this

19   technique when carried out in batch and classical methods, is the large volume of solvents used. The

20   use of flow-based platforms to carry out a LLE provides a reduction in solvent consumption as volumes

21   in the microliter range are used (Šrámková et al. 2014). The coupling of these two techniques also

22   increases throughput and the enrichment factor, decreases the possibility of contamination, and allows

23   for a more versatile method (Costa and Araújo 2001; Šrámková et al. 2014). The LLE procedure can

24   be divided into three classes: dispersive liquid-liquid extraction (DLLE), where the dispersion of fine

25   droplets of extraction solvent occurs in an aqueous sample (Quigley et al. 2016); hollow fibre liquid

26   phase extraction (HF-LPE), where a hollow fibre is immersed in the organic phase prior to the sample

27   (Astrid Gjelstad 2013); and single drop microextraction (SDME) that makes use a single drop of a few

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microliters of solvent (Kocúrová et al. 2013; Quigley et al. 2016). As reported in Table 4, this technique is not frequently used for wine analysis in flow-based platforms; however, it is commonly used in batch mode prior to analysis. In the future, the incorporation of LLE in flow-based approaches could be advantageous to carry out these complex methodologies in an automatic and easier way. With the possibility of coupling flow-based systems to a chromatograph, the automation of these sample pre-treatments would be easier to execute.

2.3. Low pressure chromatography separations

Liquid or gas chromatography are the most common separation techniques used in classical wine analysis. One of the disadvantages of these methods is that they require sophisticated, complex and costly apparatus. Although generally not providing the same efficiency, low pressure flow injection systems can be an excellent alternative (Santos and Rangel 2012) in some cases, as they provide high-performance chemical separation with low cost instrumentation (Vidigal and Rangel 2014). The choice of column and mobile phases are critical since the separation occurs in a low pressure mode (Hartwell et al. 2013). The use of monolithic columns are convenient for this type of application as they present high resolution even at lower pressure which impelled the development of new methodologies (Santos and Rangel 2012). Low pressure chromatography has been carried out in a flow injection mode (FIC, flow injection chromatography) and in a sequential injection mode (SIC, sequential injection chromatography), and these have demonstrated to be efficient mostly in the pharmaceutical industry, but also for cosmetic, food, and clinical samples (Hartwell et al. 2013). Most of the presented methods use of SIC rather than FIC and these were focused on the fast separation of relatively simple mixtures of analytes in pharmaceutical samples (Chocholouš et al. 2019). In wine analysis, only one work was published that uses a flow injection apparatus coupled to a guard chromatography column (Table 5). The system was used in the development of a methodology for the determination of the sugar and ethanol content in a fermentation process and Port wine (Vidigal and Rangel 2014).

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Low pressure chromatography is not commonly used in wine analysis due to the complexity of the sample matrix and therefore this approach is mainly used in the separation of simple mixtures such as pharmaceutical products. High or ultra-high pressure liquid chromatography might be superior in separation resolution, while low pressure have been shown to be sufficiently efficient in many separation cases (Hartwell et al. 2013). The application of this technique in wine analysis should be more feasible with the development of new columns that provide good resolution of complex mixtures at low pressure.

3. Conclusions

As referred before, wine has a very complex matrix, and several analytes of interest in terms of quality control. Thus, among the presented works, in terms of analyte of interest (Figure 3), the majority of methods were applied to the determination of sulphites (32%) coupled to membrane-based separation techniques, mainly with the gas-diffusion approach, and diverse detection systems. For this determination, all the sulphites present in the sample, free or complexed form, should be converted to sulphur dioxide (SO_2) prior to the determination. Due to the volatility of this molecule, the separation by means of a gas-diffusion membrane increases the selectivity of the determination.

Another analyte of interest was ethanol, responsible for 14% of the applications; the methods make use of the several separation techniques presented in this review. Due to its volatility, it can be separated from the matrix using gas-diffusion (Pais et al. 2013) or pervaporation (González-Rodríguez et al. 2003). Taking into consideration the polarity of this molecule, it is also possible to extract it form the sample matrix, either with liquid-liquid separation (Gallignani et al. 2005) or using a chromatographic column (Vidigal and Rangel 2014). With all of these methods, it was possible to reach low levels of LOD and LOQ. However, with gas-diffusion separation, better repeatability was attained; on the other hand, higher values of RSD were observed in the pervaporation methods.

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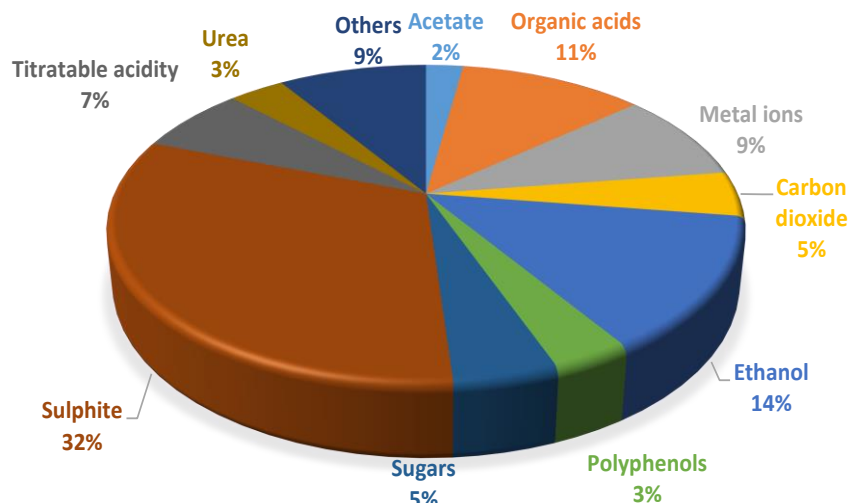
2 Figure 3 Percentage of number of papers published up to 2020 according to the analyte of interest.

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4 Interestingly, the determination of organic acids normally involves separation by dialysis. The same

5 occurs in the determination of reducing sugars. This fact may be due to the non-volatility of these

6 molecules, where dialysis plays also an important role in the sample dilution and in the elimination of

7 interfering compounds from the matrix.

8 In this scenario, flow-based systems were successfully coupled to separations techniques, to overcome

9 the problem of the possible interference of some matrix compounds in the determination of several

10 parameters in wine, due to the very complex matrix of these samples. Sample preparation can affect the

11 analyte concentration and the cleanliness of the sample prior to further analysis (Leong et al. 2014).

12 Separation techniques allow not only the clean-up of the sample, but also either a dilution, by the use

13 of membrane-based separations, or the enrichment of the analyte with the use of solid-phase extraction

14 or chromatography like techniques. This separation step prior to analysis allows the potential increase

15 on the sensitivity and repeatability of the developed methods, with a relatively low-cost solution making

16 use of simple instrumentation.

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Table 1 Analytical figures of merit of flow-based methods for wine analysis coupled to a gas-diffusion unit.

Analyte	Matrix	Flow system	Detection system	Range of applicability	LOD LOQ	Sampling rate (det./h)	RSD	Reference
Sulphite	Juice and wine sample	FIA	Amperometric	1.0 to 12 mg/L	0.26 mg/L 0.86 mg/L	40	0.4 %	(De Paula et al. 2016)
Ethanol	Red and white wine Port wine Beer Liquors, spirts brandies	FIA	Spectrophotometric	Up to 25%	0.6% 2.0%	60	< 4.6%	(Vidigal and Rangel 2015)
Sulphite	Wine samples	FIA	Voltammetric	10 to 250 mg/L	3 mg/L 9 mg/L	15	< 6%	(Gonçalves et al. 2010)
Sulphite	Red and white wines	FIA	Spectrophotometric	1.00 to 250 mg/L	0.3 mg/L 0.8 mg/L	25	1.8%	(Oliveira et al. 2009)
Sulphite	Red and white wines	FIA	Spectrophotometric	1 to 40 mg/L	0.3 mg/L 1.0 mg/L	10	2.2%	(Tzanavaras et al. 2009)
Sulphite	Fruit juices White and red wine	FIA	Spectrophotometric	1 to 200 mg/L	0.12 mg/L	40	3.3%	(Santos and Korn 2006)
Urea	Rice wine	FIA	Spectrophotometric	16 µM to 1.0 mM	ND	ND	3%	(Iida et al. 2006)
Acetic acid; sulphite	Fruit juices Red, rose and white wine	FIA	Conductimetric	0.01to 1 M 1 to 50 mg/L	5×10^{-6} M 0.03mg/L	80 120	0.8% 0.2%	(Tavares Araújo et al. 2005)
Sulphite	Fruit juices Wine	FIA	Amperometric	20 to 100 µM	2 µM	40	4.9%	(Lowinsohn et al. 2004)
Urea	Rice wine	FIA	Spectrophotometric	7.8 µM to 1.0 mM	ND	ND	ND	(Iida et al. 2003)
Sulphite	White wine	FIA	Spectrophotometric	1 to 20 mg/L	0.4 mg/L	30	0.015%	(Melo et al. 2003)
Sulphite	White and red wines	FIA	Fluorometric	40 nM to 1 Mm	ND	ND	10.5%	(Maria and Spohn 2001)
Sulphite; Ascorbic acid	Fruit juices Red and white wine	FIA	Electrochemical	0.25 to 15 mg/L 3 to 50 mh/g/L	0.05 mg/L 1.5 mg/L	30	4% 1%	(Cardwell and Christophersen 2000)
Sulphite; Carbon dioxide;	Wine samples	FIA	Spectrophotometric	0.05-0.3 g/L 0.25-3 g/L	ND	40	4.5% 2.4%	(Atanassov et al. 2000)
Sulphite	Fruit juices Wine	FIA	Bulk acoustic wave	5 to 1000 µM	1 µM	78	0.6%	(Yao and Su 1999)
Sulphite	White and red wine	FIA	Electrochemical	ND	3 µM	45	1.9%	(Azevedo et al. 1999)
Ethanol	Wine samples	FIA	Spectrophotometric	2 to 25%	0.4%	30	2.2%	(Rangel and Tóth 1999)
Sulphite	Wine samples	FIA	Conductometric	ND	1 mg/L	ND	0.8%	(Arribas et al. 2012)
Sulphite; Carbon dioxide; acetate	wine samples	FIA	CE	ND	ND	15	1.8%	(Kuban and Karlberg 1998)
Sulphite	Wine	FIA	Potentiometric	3.2 to 180 mg/L	ND	ND	75	(Araújo et al. 1998)

Sulphite	White, red and rose wine	FIA	Spectrophotometric	1 to 20 mg/L	0.1 mg/L	ND	0.7%	(Decnop-Weever and Kraak 1997)
Ethanol	Beer and wine	FIA	Amperometric	Up to 15%	0.0001%	30	ND	(Mohns and Künnecke 1995)
Sulphite	Wine and shrimp	FIA	Spectrophotometric	0.27 to 16.2 ppm	68 ppb	ND	ND	(Prieto et al. 1994)
Sulphite	Wine samples	FIA	Amperometric	0.05 to 2 mg/L	0.05 mg/L	24	2%	(Thanh et al. 1994)
Sulphite	Wine samples	FIA	Chemiluminescence	10 to 80 µM	ND	6	2%	(Huang et al. 1992)
Sulphite	Wine	FIA	Spectrophotometric	ND	ND	ND	ND	(Bartroli et al. 2002)
Ethanol	Beer, wine, spirits and medicine	FIA	Spectrophotometric	0.0006 to 60%	ND	120	ND	(Künnecke and Schmid 1990)
Sulphite	Shrimp, potatoes, dried pineapple wine	FIA	Spectrophotometric	ND	ND	ND	ND	(Sullivan et al. 1990)
Sulphite; Carbon dioxide	Wine	FIA	Potentiometric	ND	ND	25	7%	(Linares et al. 1989)
Sulphite	White and red wine	FIA	Spectrophotometric	ND	ND	90	1%	(Möller and Winter 1985)
Volatile acidity	White wine	SIA	Spectrophotometric	Up to 1.06 g/L	0.02 g/L 0.09 g/L	35	2.7%	(Vidigal and Rangel 2017)
Ethanol	Table and port wine	SIA	Spectrophotometric	up to 25%	0.004%	21	3.5%	(Pais et al. 2013)
Sulphite	White and red wine	SIA	Amperometric	0.2 to 20 mg/L	0.05 mg/L	65	1%	(Chinvongamorn et al. 2008)
Sulphite	Table wine	SIA	Spectrophotometric	2 to 250 mg/L	0.1 mg/L	16	1.2%	(Segundo and Rangel 2001)

CE – Capillary electrophoresis; ND- no data reported.

Table 2 Analytical figures of merit of flow-based methods for wine analysis coupled to a dialysis unit.

Analyte	Matrix	Flow system	Detection system	Range of applicability	LOD LOQ	Sampling rate (det./h)	RSD	Reference
Reducing sugars	Wine and liquors	FIA	Spectrophotometric	0.252 to 4.0 g/L	0.03 g/L	45	2.6%	(Da Silva et al. 2018)
Organic acids	Thai wine samples	FIA	Spectrophotometric	250 to 7500 mg/L	ND	7.5	5.4%	(Kritsunankul et al. 2009)
Tartaric acid	Table and port wine	FIA	Spectrophotometric	0.5 to 4 g/L	0.08 g/L	36	1.6%	(Silva and Alvares-Ribeiro 2002)
Malic acid	Wines	FIA	Spectrophotometric	0.05 to 1 g/L	0.03 g/L	15	2%	(Mataix and Luque De Castro 2001)
Lactic acid			Fluorometric	0.02 to 1.5 g/L	0001 g/L		2%	
				0.01 to 1 g/L	0.05 g/L		2%	
				0.05 to 1.5 g/L	0.01 g/L		2%	
Malic acid	Port and Table wine samples	FIA	Spectrophotometric	0.4 to 3 g/L	0.09 g/L	20	5%	(Lima et al. 1998)
Lactic acid					0.05 g/L			
Titratable acidity Tartaric acid	Port and table wine samples	FIA	Spectrophotometric	0.5 to 10 g/l	ND	32	3%	(Rangel and Tóth 1998)
Reducing sugars	Wine and	FIA	Spectrophotometric	1.2 to 7.2 g/L	ND	40	1.72%	(Peris-Tortajada et al. 1992)
Reducing sugars	Table and port wine	SIA	Spectrophotometric	2 to 140 g/L	1.2 g/L	18	2.1%	(González-Rodríguez et al. 2002b)
L(+)-lactate	Wine	SIA	Spectrophotometric	0.25 to 2.5 g/L	0.074 g/L	14	2%	(Araújo et al. 1997)

ND- no data reported.

Table 3. Analytical figures of merit of flow-based methods for wine analysis coupled to pervaporation units.

Analyte	Matrix	Flow system	Detection system	Range of applicability	LOD LOQ	Sampling rate (det./h)	RSD	Reference
Sulphite	Wines, vinegar, beverage and ambient air	FIA	Spectrophotometric	1 to 5 mL	0.33 mg/L	12	7.6%	(Balansay et al. 2010)
Volatile acidity	Wine	FIA	Spectrophotometric	0.20 to 0.80 g/L	0.035 g/L	10	13%	(Cuadrado et al. 2006)
Ethanol	Wine	FIA	Density meter	Up to 40%	0.11%	15	7%	(González-Rodríguez et al. 2003)
Ammonia Urea	Wine and must	FIA	Spectrophotometric	0.008 to 80 mg/L 0.15 to140 mg/L	0.6 and 0.67 mg/L 0.90 and 1.02 mg/L	16	ND	(González-Rodríguez et al. 2002a)
Volatile acidity	Wine	FIA	Spectrophotometric	0.20 to 0.80 g/L	0.032 g/L 0.086 g/L	10	ND	(González-Rodríguez et al. 2001)
Ethanol Glycerol	Red and white wine	FIA	Spectrophotometric Fluorometric	1 to 20% 2 to 8 g/L	0.5% 1.5g/L	6	3% 2%	(Mataix and Luque De Castro 2000a)
Acetaldehyde Ethyl acetate Methanol Ethanol	Red and white wine	FIA	GC-FID	20 to 100 mg/L 20 to 200 mg/L 0.02 to 0.05% 4 to 10%	12 mg/L 15 mg/L 0.02% 2%	ND	9% 4% 5% 5%	(Mataix and Luque De Castro 2000b)
Total acidity Volatile acidity	Red and white wine	FIA	Spectrophotometric	20 to 80 meq/L 0.1 to 1.5 g/L	10 meq/L 0.04 g/L	ND	1% 5.2%	(Mataix and Luque De Castro 1999a)
Carbon dioxide; Sulphite	Red and white wine	FIA	Potentiometric Photometric	50 to 600 mg/L 2.0 to 20.0 mg/L	35 mg/L 1.0 mg/L	10	7% 7%	(Mataix and Luque De Castro 1999b)
Ethanol	Musts	FIA	Fluorometric	0.01 to 0.02%	0.0004%	5	3.9%	(Delgado-Reyes, F., Papaefstathiou, I., Fernández Romero, J. M. and Luque de Castro 1998)
Sulphite	Red and white wine	FIA	Spectrophotometric	2.0 to 20 mg/L	1.2 mg/L	12	3.0%	(Mataix and Luque De Castro 1998)

GC-FID - gas chromatography-flame ionization detection; ND- no data reported

Table 4 Analytical figures of merit of flow-based methods for wine analysis coupled to extraction methods.

Analyte	Matrix	Flow system	Detection system	Range of applicability	LOD; LOQ	Sampling rate (det./h)	RSD%	Extraction technique	References
Lead	Red Wine, water and baby food	FIA	FAAS	5.0 to 15.0 µg/L	0.90 µg/L	ND	< 4%	SPE - Coliform bacteria immobilized on TiO_2 nanoparticles	(Bakircioglu et al. 2011)
Lead	Wine, blood, human hair, water	FIA	HG-AFS	0.02 to 2.0 µg/L	4 µg/L	50	1.6%	SPE - Iminodiacetate chelating resin beads	(Wan et al. 2006)
Cadmium Copper Lead	Wine	FIA	AAS	ND	2.0 µg/L 1.6 µg/L 11 µg/L	ND	2% 2% 6%	SPE - C-18 bonded silica gel and powdered polyethylene (PE)	(Pires Fernandes et al. 2003)
Lead	Table and port wine; Water	FIA	FAAS	0.5 to 15 µg/L	6 µg/L	24	ND	SPE - Pb-Spec®	(Bakircioglu et al. 2003)
Anthocyanins MV; CY; PE	Table wine	FIA	Spectrophotometric	1.0 to 60 mg/L 0.5 to 16 mg/L 0.5 to 16 mg/L	0.2 mg/L 0.2 mg/L 0.2 mg/L	ND	10% 10% 15%	SPE - C18 minicolumn for matrix removal and preconcentration prior to HPLC	(Mataix and Luque de Castro 2001)
Biogenic amines	Table wine	FIA	CE	0 to 10 mg/L	0.05 to 0.1 mg/L	2	ND	SPE - C18 minicolumn for sample clean-up and preconcentration prior to CE	(Arce et al. 1998a)
Resveratrol Polyphenols	Table wine	FIA	CE	0.05 to 100 mg/L	0.05 to 0.36 mg/L	50	3.2 to 7.1%	SPE - C18 minicolumn for sample clean-up and preconcentration prior to CE	(Arce et al. 1998b)
Protein	White wine, sparkling wine and beer	LOV	Spectrophotometric	Up to 0.30 g/L	0.03 g/L 0.10 g/L	9	< 5%	SPS - NTA superflow resin coupled to Cu^{2+}	(Vidigal et al. 2012)
Quercetin	Red wine and urine	LOV	Voltammetry	0.01 to 10 µM	0.0013 µM	40	2.9%	SPE - Octadecyl functionalized magnetic silica nanoparticles	(Wang et al. 2012)
Iron	Table and Port wine; Brines	LOV	Spectrophotometric	0.09 to 5.0 mg/L	0.02 mg/L 0.09 mg/L	20	ND	SPS - NTA superflow resin	(Vidigal et al. 2011)
Ethanol	Table wine Spirits and beer	FIA	FTIR	Up to 15%	0.03% 0.1 %	25	1.3%	LLE with chloroform	(Gallignani et al. 2005)
Ethanol	Table Wine	SIA	Spectrophotometric	Up to 1.5%	0.025%	12	< 4%	HS-SDME with potassium dichromate	(Šrámková et al. 2014)
Iron	Table Wine	SIA	FAAS	1.5 to 15 mg/L	0.03 mg/L 0.10 mg/L	ND	< 5%	LLE with methylisobutylketone	(Costa and Araújo 2001)

AAS - atomic absorption spectrometry; CE – Capillary electrophoresis; CY - cyanidin-3-glucoside; FAAS – Flame atomic absorption spectrometry; HG-AFS – Hydride generation atomic fluorescence spectroscopy: HS-SD-ME – Head-space Single-drop Micro-extraction; LLE – Liquid-liquid extraction; MV - Malvidin-3-glucoside; ND- no data reported; PE - peonidin-3-glucoside; SPE – Solid phase extraction; SPS – Solid phase spectrometry.

Table 5 Analytical figures of merit of flow-based methods for wine analysis coupled to low pressure chromatography separation methods.

Analyte	Matrix	Flow system	Detection system	Range of applicability	LOD; LOQ	Sampling rate (det./h)	RSD%	Column	References
Sugars Ethanol	Fermentation broth Port wine	FIA	Spectrophotometric	Up to 12 g/L Up to 2%	2.3 g/L 0.4%	30	4%	Guard cation H^+ cartridge	(Vidigal and Rangel 2014)

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