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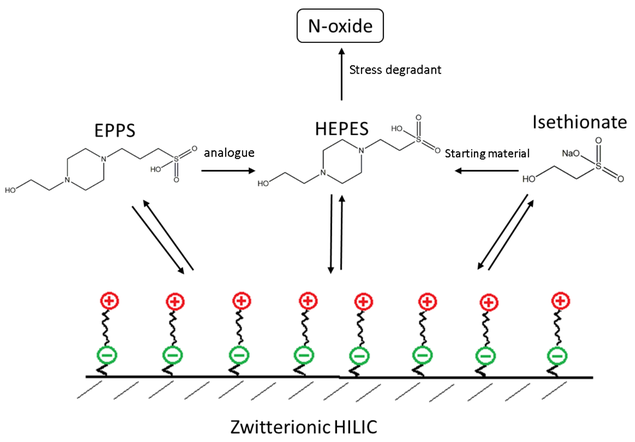
Hydrophilic interaction liquid chromatography method development and validation for the assay of HEPES zwitterionic buffer

Xiaolong Xu, Bert Gevaert, Nathalie Bracke, Han Yao, Evelien Wynendaele, Bart De Spiegeleer*

Drug Quality and Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium

*Corresponding author: bart.despiegeleer@UGent.be (*O. Ref.*: 2016-363b)

INTRODUCTION



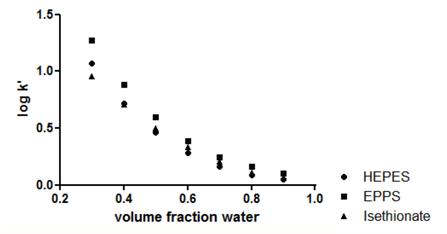
- **HEPES**: zwitterionic buffer, raw material in the GMP-manufacturing of **ATMPs** (gen-, cell- and tissue medicinal products)
  => Must: adequate assay method with sufficient selectivity towards related impurities
  However: formal GMP-compliant quality control methods are currently lacking.
- **HILIC:** Analysis of **polar compounds** which are weakly retained on RP-LC.
  Zwitterionic HILIC: dual charged hydrophilic stationary phase

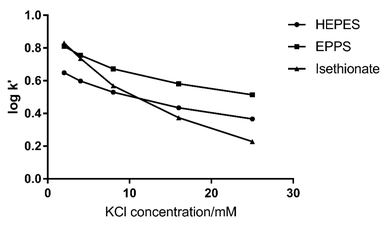
EXPERIMENTAL

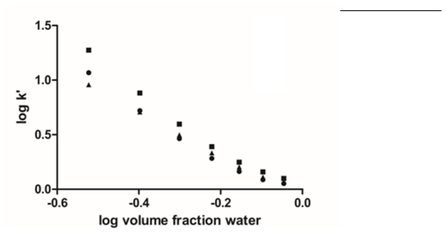
- **Method development**: the influence of column temperature, solvent strength, pH and ion concentration of mobile phase on the retention of target compounds was investigated.
- **Final method**: stationary phase: Obelisc N column (3.2 × 150 mm, 5 µm, SIELC Technologies, series: ONNX70UD). UV detection at 195 nm. Mobile phase: 35/65 V/V water (adjusted to pH 2.0 with H_3PO_4) and acetonitrile. Flow rate: 0.5 mL/min. Column temperature: 30°C. Injection volume: 10 µL.
- **Modelling the retention**: four different retention models were applied to the retention data.
- **Stress testing and identification of degradant (LC-MS)**
- **Method validation**

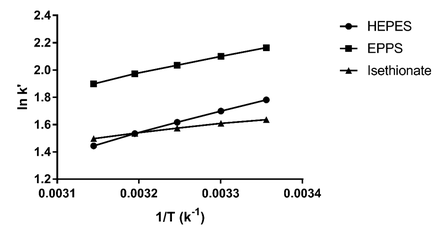
RESULTS and DISCUSSION

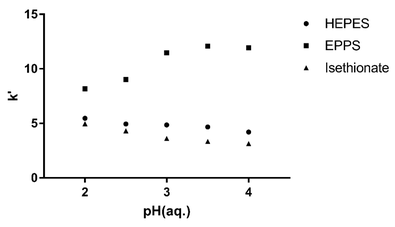
1. Method development











2. Modelling of retention

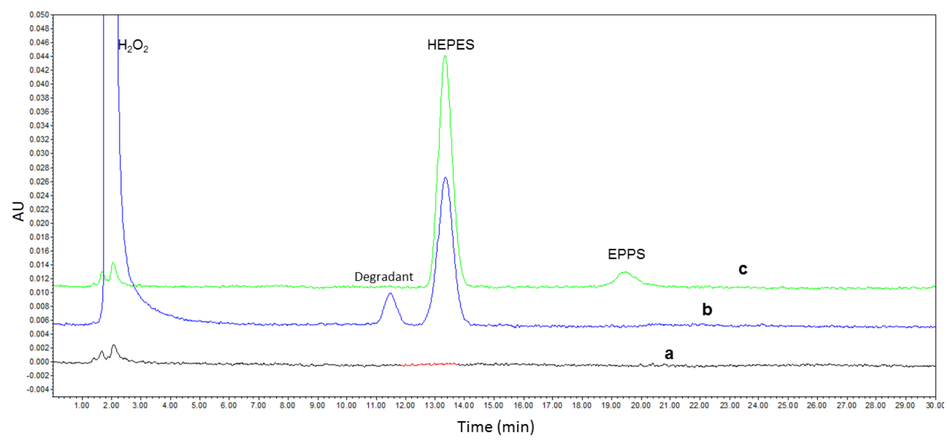
	Partition model			Adsorption model			Mixed model				Ion exchange mode		
	log k_0 ± SE	m ± SE	R^2	log k_0 ± SE	m ± SE	R^2	a	m_1	m_2	R^2	S ± SE	a ± SE	R^2
HEPES	1.394 ± 0.153	1.647 ± 0.241	0.9032	0.134 ± 0.046	2.171 ± 0.157	0.9746	-1.722 ± 0.121	1.742 ± 0.133	4.353 ± 0.168	0.9994	0.258 ± 0.021	0.743 ± 0.020	0.9809
EPPS	1.661 ± 0.163	1.900 ± 0.258	0.9156	0.099 ± 0.046	2.495 ± 0.155	0.9811	-1.676 ± 0.095	1.729 ± 0.104	4.662 ± 0.132	0.9997	0.273 ± 0.016	0.907 ± 0.016	0.9894
Isethionate	1.304 ± 0.098	1.488 ± 0.154	0.9490	0.070 ± 0.020	1.932 ± 0.068	0.9939	-0.662 ± 0.182	0.649 ± 0.199	2.746 ± 0.252	0.9983	0.556 ± 0.046	1.038 ± 0.044	0.9802

- The partition and adsorption mechanism both contributing the retention of the analytes at the applied column
- Adsorption mechanism playing a more important role
- Ion exchange mechanism also existing for this column

3. Stress testing and identification of degradant

No degradation product was formed except during oxidative stressing. Under oxidative stress conditions, a degradant peak at Rt 11.48 min was observed, which is well separated from the HEPES peak at Rt 13.35 min. High resolution MS data of the degradant indicated that the formula $[M+H]^+$ was $C_8H_{19}N_2O_5S$ (experimental mass 255.1002; calculated mass 255.1015, error -1.3 mDa). It complies with the previous data that Good's buffers containing morpholine or piperazine rings like HEPES can be oxidized to their N-oxide forms.

3. Method validation



Linearity:
The standard curve was linear over the range of 0.5 mg/mL, with a R square equally to 0.999.

Precision and accuracy:

Concentration (mg/mL)	N	Precision (RSD%)	Accuracy (recovery%)
0.4 (80% level)	3	0.088	100.24
0.5 (100% level)	3	0.510	99.92
0.6 (120% level)	3	0.265	100.10

4. Assay of HEPES in commercial samples

The developed method was applied for the assay determination of HEPES products obtained from three different supplier on the market. The obtained content of HEPES was 99.88% (95% CI: [99.78%-99.98%]).

CONCLUSION

- A HILIC method for the analysis of HEPES was developed, validated and applied to commercial products from different suppliers.
- The influence of different factors on retention time was investigated to get a better understanding of the retention.
- The obtained data were evaluated by different retention models and a mixed model of partition and adsorption mechanism was found to fit best, with adsorption being the main retention mechanism, combined with partition and ion exchange mechanisms.
- The stress test of HEPES found one degradant under oxidative conditions, identified as N-oxide by high resolution MS.

REFERENCES

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