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Chromatographic analysis of ivabradine on polar, nonpolar and chemically modified adsorbents by HPTLC

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ABSTRACT

Thin-layer chromatography (TLC) was used to evaluate the behavior of ivabradine in various chromatographic systems. The mobile phases used were aqueous (methanol–water and acetonitrile–water) and non-aqueous (methanol–acetonitrile and methanol–dimethyl sulfoxide) in a wide range of concentrations (0–100%, v/v). The stationary phases used were high-performance TLC plates: Kieselgel 60 $F_{254\ s}$, RP-2 $F_{254\ s}$, RP-8 $F_{254\ s}$, RP-18 $F_{254\ s}$, RP-18 $WF_{254\ s}$, Kieselgel 60 CN $F_{254\ s}$, Kieselgel 60 Diol $F_{254\ s}$, and Kieselgel 60 NH_2 F_{254} s; and TLC plates: aluminum oxide 150 F_{254}, cellulose F, and polyamide 11 F_{254}. The usefulness of various mobile and stationary phases in the separation of ivabradine was demonstrated. Retention mechanisms as well as the Soczewiński-Snyder equation are discussed.

1. Introduction

Currently, commercially available thin-layer chromatography (TLC) plates have very high quality, but which available solvents (organic or buffered) to choose as mobile phases constitutes the greatest difficulty in optimizing chromatographic systems. The purpose of this study was to select an appropriate TLC system for the qualitative analysis of ivabradine. The method of trial and error is the only reliable way for determining the proper choice of the stationary and mobile phases, not only for TLC, but also for other chromatographic methods [1].

Ivabradine (Fig. 1) is the first modern drug synthesized to selectively decrease heart rate. Ivabradine acts by selective inhibition of the I_f (pacemaker current) in sinus node cells. Ivabradine absorption is almost complete in the case of oral administration. Maximum plasma concentration occurs approximately 1 hour after dosing on an empty stomach. The absolute bioavailability of tablets is approximately 40% due to the first-pass effect in the gut and liver [2]. Ivabradine has been analyzed by high-performance liquid chromatography (HPLC) [3–6].

One of the most well-known processes occurring during the chromatographic process is adsorption, which is described by the Soczewiński-Snyder equation [7]:

$$\log k = const - m\log X_S \quad (1)$$

where $\log k$ is the logarithm of the retention factor, *const* is the adsorption constant of the analyte to the stationary phase, m is related to the number of adsorbed functional groups in the analyte, and X_s is the molar fraction of the more polar component of the mobile phase.

The Soczewiński-Snyder competitive adsorption model assumes the competitiveness of the more polar component of the mobile phase and solute for a place on silanol groups in the stationary phase [7,8].

In this study, several attempts were made to quantify the phenomena responsible for the process of chromatography,

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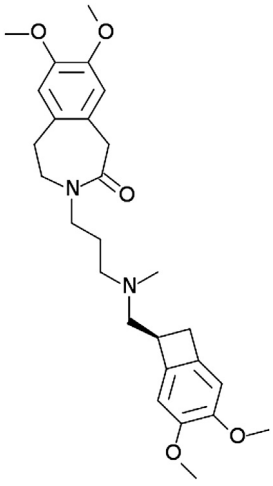

Fig. 1 – The chemical structure of ivabradine.

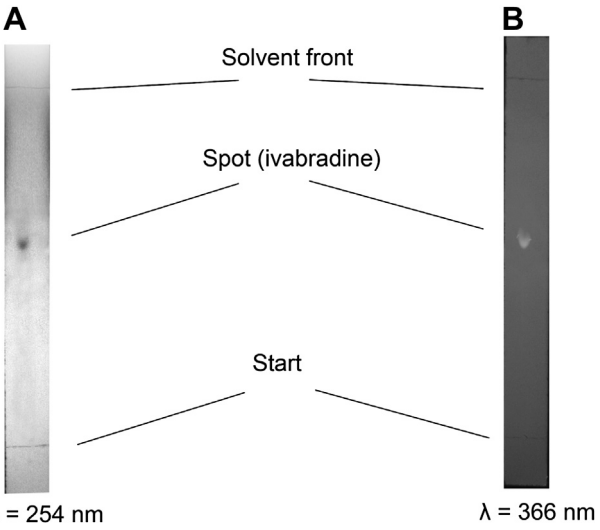

Fig. 2 – Typical chromatogram of ivabradine. Stationary phase: Kieselgel 60 $F_{254\ S}$. Mobile phase: acetonitrile–water (60:40, v/v). Detection wavelength: (A) 254 nm; (B) 366 nm.

optimum applications and further studies of these phenomena, as well as the development of a high-performance TLC (HPTLC) method for the determination of ivabradine.

2. Methods

Ivabradine hydrochloride was supplied by Watson International Ltd. (Kunshan, China). HPLC-grade methanol, acetonitrile and dimethyl sulfoxide (DMSO) were supplied by Sigma-Aldrich Co. Ltd. (Gillingham, Dorset, UK).

Fourier transform infrared spectroscopy (FTIR) was used to ensure that there were no impurities present in the compound and the FTIR spectrum was consistent with literature data [9].

Ivabradine hydrochloride was dissolved in methanol to make a stock solution at a concentration of 6 mg/mL. Binary mobile phases were prepared in a wide range from 0% to 100% (v/v) by mixing two reagents in adequate ratio by 10%.

The stationary phases were: HPTLC plates—Kieselgel 60 $F_{254\ S}$, RP-2 $F_{254\ S}$, RP-8 $F_{254\ S}$, RP-18 $F_{254\ S}$, RP-18 $WF_{254\ S}$, Kieselgel 60 CN $F_{254\ S}$, Kieselgel 60 Diol $F_{254\ S}$, and Kieselgel 60 NH2 $F_{254\ S}$; and TLC plates—aluminum oxide 150 F_{254}, cellulose F, and polyamide 11 F_{254} manufactured by Merck (Darmstadt, Germany). Cylindrical glass chambers (70 mm wide and 110 mm high) were used to develop the plates. The chamber was saturated with mobile phase steam through an impregnated paper. TLC development was conducted at room temperature (20 °C). To visualize the examined compounds which were seen as violet spots, a two-range UV lamp (254 nm and 366 nm) was used (Fig. 2).

3. Results and discussion

Maximum values for R_f were obtained after the application of DMSO as an additive in the methanol mobile phase. DMSO blocked the active sites of cellulose and alumina in the widest range of concentrations in the binary phase, containing methanol and the above-mentioned modifier. Noteworthy is the lack of interaction of DMSO with the cyano-bonded stationary phase (low R_f values for a wide range of concentrations in the mobile phase of methanol/DMSO). This is all the more interesting because DMSO generally causes an increase in elution of the analyte, in most cases leading to the presence of a minimum retention.

Water, as a modifier of methanol and acetonitrile, resulted in different chromatographic behavior of the test substance; the elution strength of the mixture of acetonitrile/water mobile phase proved to be more powerful than methanol/water (Fig. 3). From a practical point of view, for further study based on TLC, binary mobile phases containing 10–40% v/v water in acetonitrile may be recommended. In the studied concentration range, the eluent composed of methanol and water eluted ivabradine very well in the NH_2-bonded stationary phase. Average R_f values of ivabradine on cellulose and polyamide were obtained using a methanol–water mixture, which allowed further analysis with these adsorbents and mobile phase in the whole range of concentrations.

Comparing the results from the study after the application of non-aqueous methanol/acetonitrile, it should be noted that the mobile phase eluted ivabradine slightly on silica gel. However, when cellulose and polyamide were used as stationary phases, high R_f values of ivabradine were obtained. Plates RP-2, RP-8, RP-18 and a plate coated with aluminum oxide may be recommended for further analysis in a wide range of concentrations of methanol in acetonitrile. For sorbents, in which the surface of the silica gel is linked with alkyls having 2, 8 and 18 carbon atoms in the molecule, it was observed that with increasing alkyl chain length modifying the silica gel, elution with methanol–acetonitrile decreased and the maximum shifted the elution towards the reduction of methanol in acetonitrile.

Regression and correlation coefficients (r) obtained for ivabradine are presented in Table 1. Non-linear behavior suggests the occurrence of multiple types of retention mechanism or the presence of multiple types of binding site.

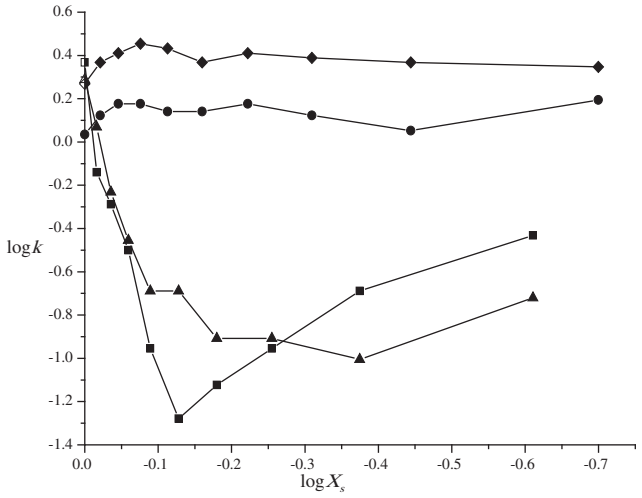

Fig. 3 – Log k values of ivabradine plotted against the logarithm of the mole fraction of the organic modifier (log X_s) in the mobile phase. –●– = mobile phase of water–methanol and stationary phase of polyamide 11 F_{254}; –◆– = mobile phase of water–methanol and stationary phase of cellulose F; –■– = mobile phase of water–acetonitrile and stationary phase of polyamide 11 F_{254}; –▲– = mobile phase of water–acetonitrile and stationary phase of cellulose F.

Changes in conformation and changes in the extent to which the mobile phase interacts with either ivabradine or the stationary phases also lead to non-linearity in plots. The polarity of cyano- and diol-bonded phases is smaller than the polarity of silica, and greater than the *octadecyl phase*. Therefore, the two above-mentioned modified stationary phases, depending on the eluent used, behave as normal or reversed phase systems. Table 2 [10–12] shows the values of ionization potential, dipole moment and polarizability of methanol and DMSO. A mixture of both solvents is considered to be polar, and can therefore successfully elute polar substances chromatographed in reversed phase.

DMSO led to a substantial decrease in the retention of ivabradine. This could be explained by the assumption that DMSO (ionic form) occupied adsorption centers on the silica surface not covered by the impregnating agent, resulting in reduced retention capacity. This finding also indicates that the addition of DMSO to the mobile phase considerably modified the retention behavior of these compounds in reversed-phase chromatography. The acidic character of the support (presence of free silanol groups) also prevailed even after coating and allowed the binding of compounds containing basic substructures. It is known that in reversed-phase TLC, the support partially retains its original adsorption characteristics even after coating. The role of the free silanol groups prevailed at higher organic ratio. This silanophil effect could be eliminated by adding the modifier (DMSO) to the eluent, the ionic forms of which bind to the remaining adsorption sites of the coated silica, neutralizing them or decreasing their influence on retention and suppressing the dissociation of polar substituents. The acidic character of the support was then reduced.

Table 1 – Linear and non-linear behavior of ivabradine: regression coefficients (*a*, *b*) and correlation coefficients (*r*) for the regression equation log $k = aS[\%] + b$ for ivabradine in binary mobile phases.[a]

Mobile phase	Stationary phase	*a*	SD	*b*	SD	*r*	*n*
Methanol–water	Sillica gel	−0.011	0.0012	1.899	0.0761	−0.967	9
	Aluminum oxide	0.002	0.0008	−0.790	0.0579	0.807	7
	CN	−0.011	0.0028	2.018	0.1740	−0.813	10
	Diol	−0.008	0.0032	1.259	0.1896	−0.639	11
Acetonitrile–water	Aluminum oxide	0.023	0.0033	−2.010	0.2425	0.952	7
	CN	−0.013	0.0055	1.381	0.3439	−0.652	10
	Cellulose	−0.010	0.0023	−0.021	0.1418	−0.820	11
Methanol–acetonitrile	Sillica gel	−0.009	0.0023	1.328	0.1426	−0.819	10
	Aluminum oxide	−0.008	0.0019	0.067	0.1150	−0.808	11
	RP-8	0.019	0.0066	−0.676	0.409	0.714	10
	NH_2	−0.007	0.0027	−0.177	0.1589	−0.663	11
	CN	0.008	0.0031	0.348	0.1849	0.655	11
	Cellulose	0.009	0.0028	−1.070	0.1681	0.726	11
Methanol–DMSO	Sillica gel	0.023	0.0037	−2.140	0.2299	0.915	10
	RP-2	0.028	0.0085	−2.401	0.5295	0.762	10
	RP-8	0.041	0.0089	−2.982	0.6023	0.886	8
	RP-18	0.023	0.0039	−2.164	0.2593	0.912	9
	NH_2	0.005	0.0018	−1.208	0.1064	0.732	11
	CN	0.008	0.0024	0.303	0.1567	0.781	9
	Diol	0.033	0.0037	−2.015	0.2554	0.965	8
	Polyamide 11	0.010	0.0041	−1.429	0.2394	0.649	11

a Only results with $p < 0.05$ are presented. S[%] = ratio of more polar solvent in less polar in percent; SD = standard deviation; *n* = number of variables used in the calculation.

Single-point adsorption combined with the dissociation of the solvation shell consisting of DMSO is explained by the slope (Table 3) of the Soczewiński-Snyder equation curve in the range from 2 to 1 (silica gel, aluminum oxide, RP-18). However, for the slope greater than 2 (RP-8 and diol), it is more likely that multi-point adsorption is also connected to the dissociation of the solvation shell. Taking into account the H-bonding properties of NH2 and CN groups, as well as the low absolute values of the slope, a more complex mechanism of retention for these stationary phases is assumed [7,8,13]. Data obtained using the Soczewiński-Snyder equation suggested the formation of a solvation shell consisting of DMSO.

A polyamide adsorbent is expected to provoke multiple effects affecting the retention mechanism: partition, sieve effect and/or formation of hydrogen bonds. In the case of the amino-bonded stationary phase, the formation of hydrogen

Table 2 – Ionization energy (IP), dipole moment (μ) and polarizability (α) of the components in the binary mobile phases.

	IP (eV) [10]	μ (D) [11]	$\alpha \times 10^{-24}$ (cm^3) [12]
Methanol	10.85	1.7	3.29
Acetonitrile	12.2	3.92	4.4
Water	12.6	1.85	1.45
DMSO	9.1	3.96	7.99

Table 3 – Parameter values of the Soczewiński-Snyder equation for ivabradine and methanol–DMSO as the mobile phase.[a]

	Slope	SD	Intercept	SD	*n*	*r*	*p*
Silica gel	−1.842	0.2572	−2.043	0.1945	6	−0.963	0.002
Aluminum oxide	−1.481	0.2123	−2.561	0.1877	4	−0.978	0.022
RP-2	−1.836	0.5201	−2.334	0.3313	9	−0.795	0.010
RP-8	−2.865	0.4837	−2.402	0.3207	8	−0.924	0.001
RP-18	−1.467	0.2233	−1.754	0.1481	8	−0.937	0.0005
NH_2	0.619	0.1382	−0.829	0.0475	6	0.913	0.011
CN	−0.578	0.1159	0.318	0.0877	6	−0.928	0.007
Diol	−3.034	0.5467	−1.726	0.3150	6	−0.941	0.005

a For the analyzed chromatographic systems for the mobile phase consisting of methanol–DMSO and stationary phases of cellulose and polyamide, *p* was greater than 0.05 and therefore not included in the table. SD = standard deviation.

bonds may also occur [1,14]. With the presence of relevant basic functional groups such as amide groups and aminopropyl-bonded groups on the surface of sorbents, the formation of hydrogen bonds with ivabradine occurred (Fig. 3).

4. Conclusion

The chromatographic process occurring on the amino-bonded stationary phase is conditioned primarily on the properties of the sorbent. However, the physicochemical parameters of the eluent did not significantly affect the retention behavior of ivabradine, as for the application of cyano- and diol-bonded stationary phases. The nature of the solvent mixtures (aqueous or non-aqueous) with different elution strengths had no effect on the value of the log *k* parameter.

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