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Adsorption of drugs in high-performance liquid chromatography injector loops

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ABSTRACT

The effect of solvent injection on the area and peak-height responses of some drugs (ergotamine tartrate, astemizole, terfenadine, bromhexine hydrochloride, caffeine, ambroxol hydrochloride, phenylephrine hydrochloride, enalapril maleate and betamethasone) was evaluated. Reversed-phase chromatographic systems were employed and loops of different sizes and various overfill volumes were assayed. When injection solvents weaker than the mobile phases were used, significant variation in the area responses was observed for astemizole, bromhexine, ergotamine and terfenadine. Adsorption on the internal surface of the injection loops produced this anomalous behaviour depending on the chemical nature of the analyte injected.

INTRODUCTION

Much attention is nowadays focused on the validation of analytical methods for drug determination in complex matrices such as biological fluids and pharmaceutical dosage forms. These analyses require compliance with good laboratory practices (GLPs) and standard operating procedures (SOPs), which dictate in detail the different validation steps of an analytical process. The applicability of HPLC in pharmaceutical analyses is well known and so the procedures used must be fully validated.

A variable to be investigated is the injection system because inaccuracy and lack of reproducibility are often caused by the sample injector. Quantitation problems in measuring peak height or area arising from sample–solvent interaction phenomena [1–9] or adsorption effects onto the injection system [10–13] have been reported. This adsorption is observed particularly when the solvent used is water or weaker than the mobile phase in RP-HPLC.

Taking into account the adsorption effects, Dolan [11] reported different inter-laboratory results obtained in the analysis of an antihistaminic agent because drug adsorption in the injector loop caused increased area responses related to the variable overfill volume. Similar results were found when samples of aqueous nonylphenol ethoxylate surfactant solutions were injected [12]. Inaccuracies and reproducibility problem have also been observed on injection of aqueous solutions of vinblastine and vancomycin, and this has been attributed to their interaction with the injection system [7].

MacLeod *et al.* [10] suggested that the valve rotor caused adsorption of aqueous solutions of an ansiolitic agent, and Simonson and Nelson [13] demonstrated amitriptyline adsorption on the inner wall of the injection loop.

Because of drug regulations, *in vitro* dissolution studies are becoming relevant for testing the bioequivalency of pharmaceutical formulations. These assays use aqueous media and HPLC is

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often the method choice because of its selectivity and sensitivity. Therefore, we considered it of interest to study several drugs with different physicochemical characteristics at present not reported in order to investigate their anomalous response in the injection process.

EXPERIMENTAL

Reagents

Ergotamine tartrate, terfenadine, bromhexine hydrochloride, caffeine, ambroxol hydrochloride, phenylephrine hydrochloride and betamethasone were purchased from Sigma (St. Louis, MO, USA). Astemizole was from Janssen (Denmark) and enalapril maleate was from Merck Sharp & Dohme (USA).

HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Heptanesulphonic acid sodium salt was bought from Sigma and potassium dihydrogenphosphate from J.T. Baker (Phillipsburg, NJ, USA). Deionized, double-distilled water was used. Eluents were filtered through a 0.2-μm membrane filter and degassed before use.

Instrumentation

HPLC was carried out with a Varian Model 5020 liquid chromatograph equipped with a Varian UV-100 detector. Data were processed with a Varian 4270 integrator (Varian, Palo Alto, CA, USA). A Rheodyne Model 7125 injector (Cotati, CA, USA) with 20- and 50-μl loops was used. Detection was performed at 276, 254, 246 and 230 nm according to the absorption spectra of the drugs under study and at 0.05 AUFS. The columns used were a 150 × 4.6 mm I.D., 5 μm, MicroPak MCH-5 (Varian) and a 300 × 3.9 mm I.D., 10 μm, μBondapak Phenyl (Waters, Milford, MA, USA).

Solutions

Stock drug solutions of astemizole, terfenadine, caffeine and betamethasone in concentrations ranging from 0.2 to 1.0 mg/ml were prepared in methanol. Stock drug solutions of ergotamine tartrate, phenylephrine hydrochloride, bromhexine hydrochloride, ambroxol hydrochloride and enalapril maleate were prepared in water in concentrations from 0.2 to 1.2 mg/ml.

Working standard solutions were obtained from stock solutions by dilution in water, mobile phase or methanol. The final concentrations were: phenylephrine hydrochloride 19.2 μg/ml, ergotamine tartrate 34.0 μg/ml, bromhexine hydrochloride 20 μg/ml, betamethasone 20 μg/ml, ambroxol hydrochloride 24 μg/ml, enalapril maleate 10 μg/ml, astemizole 20 μg/ml, terfenadine 5 μg/ml and caffeine 30 μg/ml.

Analyses were performed by replicate injections ($n = 4$) of all drug solutions.

RESULTS AND DISCUSSION

In this report we present and discuss the results obtained in the study of the adsorption effects of some drugs on the inner wall of HPLC injector loops. Basic drugs such as phenylephrine hydrochloride, ergotamine tartrate, bromhexine hydrochloride, ambroxol hydrochloride, astemizole and terfenadine were chosen for this study. Caffeine, betamethasone and enalapril maleate were also investigated.

In our experiments a loop of 20 μl was overfilled with 60, 150 and 300 μl of aqueous solutions of the drugs under study. Statistical analysis was performed and a significant increase in the area response ($P < 0.05$) was observed depending on the degree of overfill volume used in the injection (Table I). The results obtained may be attributed to a sample adsorption mecha-

TABLE I

OVERFILL VOLUME EFFECT FOR AQUEOUS SOLUTION INJECTIONS IN A 20-μl LOOP

For chromatographic conditions, see Figs. 1, 2, 3 and 4.

Drug	Area (±S.D.)[a]		
	60 μl	150 μl	300 μl
Astemizole	12155 ± 385	16338 ± 485	17509 ± 418
Bromhexine	19397 ± 623	21725 ± 550	24548 ± 416
Ergotamine	28789 ± 788	31155 ± 584	33802 ± 664
Terfenadine	5118 ± 175	6413 ± 203	7412 ± 213

[a] Mean area (±S.D.) for four injections.

nism and are in agreement with reports of other workers [11–13]. When a strong solvent is used as mobile phase, it flows through the loop, desorbs the retained substance and, consequently, the measured areas increase.

When methanol or mobile phase was used as the injection solvent (Table II) the area response values remained constant and were not affected by the overfill volume because the injection solvents employed are strong enough to prevent drug adsorption (Figs. 1–4). Lack of an adsorption effect might be interpreted in terms of the relative free energies of adsorption and that of solubility. With methanol and mobile phase as injection solvents the free energy of solubility could be favoured [10]. No variations in the mean area responses were obtained with phenylephrine hydrochloride, caffeine, betamethasone, ambroxol hydrochloride and enalapril maleate.

In order to investigate if an adsorption process could be produce on the internal surface of the loop, aqueous solutions of astemizole, bromhexine hydrochloride, ergotamine tartrate and terfenadine were injected in 20- and 50-μl loops with a constant overfill volume of 150 μl of each solution. A comparison of area responses obtained with water and mobile phase is shown in Table III. Differences in the measured areas between the solvents for each loop are evident, and these differences are proportional to the contact surface each injected solution ran through. Our results indicate that adsorption

TABLE II

OVERFILL VOLUME EFFECT FOR MOBILE PHASE SOLUTION INJECTIONS IN A 20-μl LOOP

For chromatographic conditions, see Figs. 1, 2, 3 and 4.

Drug	Area (±S.D.)[a]		
	60 μl	150 μl	300 μl
Astemizole	12768 ± 213	12922 ± 240	12773 ± 204
Bromhexine	19859 ± 189	20003 ± 250	19920 ± 198
Ergotamine	28808 ± 237	28960 ± 207	28911 ± 287
Terfenadine	5072 ± 112	5078 ± 98	5018 ± 124

[a] Mean area (±S.D.) for four injections.

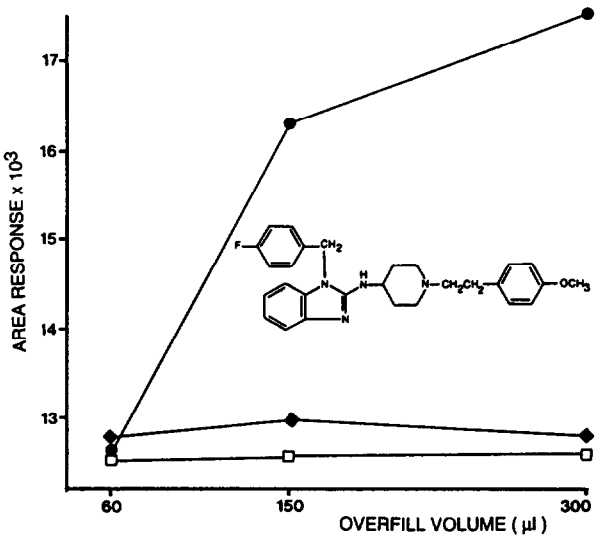

Fig. 1. Astemizole: area response as a function of overfill volume. Injection solvent: ● = water; ◆ = mobile phase; □ = methanol. Column: MicroPack MCH-5. Mobile phase: methanol–phosphate buffer 0.03 *M*, pH 3.0 (85:15). Flow-rate: 1.5 ml/min. Detection: 276 nm, 0.05 AUFS.

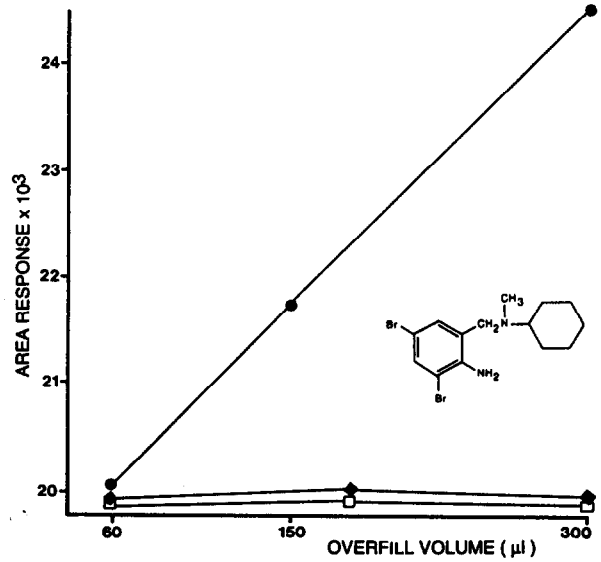

Fig. 2. Bromhexine hydrochloride: area response as a function of overfill volume. Injection solvent: ● = water; ◆ = mobile phase; □ = methanol. Column: MicroPack MCH-5. Mobile phase: methanol–phosphate buffer 0.03 *M*, pH 3.0 (85:15). Flow-rate: 1.5 ml/min. Detection: 246 nm, 0.05 AUFS.

TABLE III

AREA RESPONSES IN 20- AND 50-μl LOOPS USING WATER AND MOBILE PHASE AS INJECTION SOLVENT FOR A 150-μl OVERFILL VOLUME

For chromatographic conditions, see Figs. 1, 2, 3 and 4.

Drug	Area (±S.D.)[a]			
	Loop 20 μl		Loop 50 μl	
	Water	Mobile phase	Water	Mobile phase
Astemizole	16338 ± 485	12922 ± 240	42806 ± 1229	32305 ± 495
Bromhexine	21725 ± 550	20003 ± 250	53383 ± 990	50010 ± 508
Ergotamine	31155 ± 584	28960 ± 207	75395 ± 1100	72590 ± 475
Terfenadine	6413 ± 203	5078 ± 98	15008 ± 560	12580 ± 209

[a] Mean area (±S.D.) for four injections.

phenomena take place mainly on the inner walls of the injection loops and that drug interaction with the polymeric material of the rotor would not be the only cause of the increased areas observed. If this were the case, at constant overfill volume the area differences between the injections in water and mobile phase would be the same for the two loops used.

Basic drugs that showed adsorption phenomena were slightly soluble in water but more soluble in methanol. Our results are in agreement with those of Zlatkis and Ranatunga [14], who pointed out that the lower the solubility and

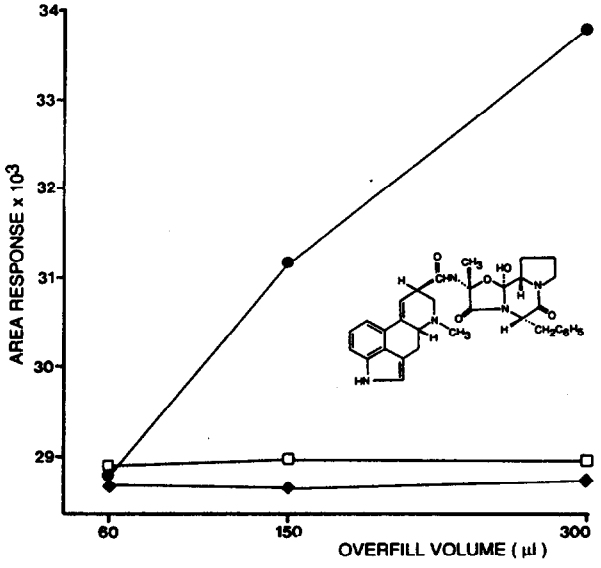

Fig. 3. Ergotamine tartrate: area response as a function of overfill volume. Injection solvent: ● = water; ◆ = mobile phase; □ = methanol. Column: μBondapak Phenyl. Mobile phase: acetonitrile–heptanesulphonic acid 1.25 m*M* in acetic acid 0.1%, pH 3.25 (65:35). Flow-rate: 1.3 ml/min. Detection: 254 nm, 0.05 AUFS.

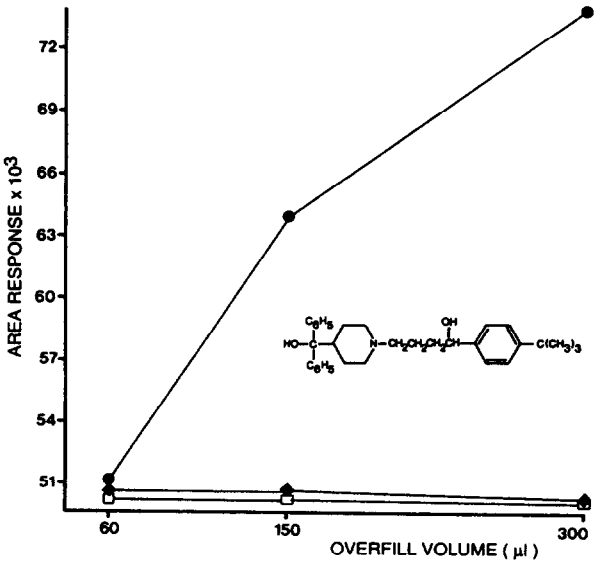

Fig. 4. Terfenadine: area response as a function of overfill volume. Injection solvent: ● = water; ◆ = mobile phase; □ = methanol. Column: MicroPak MCH-5. Mobile phase: acetonitrile–phosphate buffer 0.03 *M*, pH 3.0 (70:30). Flow-rate: 1.7 ml/min. Detection: 230 nm, 0.05 AUFS.

polarity of a compound the better it is adsorbed on the surface of a capillary tubing.

In brief, inaccuracy and lack of reproducibility in the results of HPLC may be caused by adsorption phenomena in the injector loop. This problem can be overcome if a constant overfill volume or injection solvents that favour the solubility of the drugs are used.

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REFERENCES

1 M. Tsimidou and R. Macrae, *J. Chromatogr.*, 285 (1984) 178.
2 Ch. Yi, J.L. Fasching and P.R. Brown, *J. Chromatogr.*, 352 (1986) 221.
3 S. Perlman and J.J. Kirschbaum, *J. Chromatogr.*, 357 (1986) 39.
4 J.J. Kirschbaum and S. Perlman, *J. Chromatogr.*, 369 (1986) 269.
5 K.C. Chan and E.S. Yeung, *J. Chromatogr.*, 391 (1987) 465.
6 N.E. Hoffman, S. Pan and A.M. Rusium, *J. Chromatogr.*, 465 (1989) 189.
7 E.L. Inman, A.M. Maloney and E.C. Richard, *J. Chromatogr.*, 465 (1989) 201.
8 N.E. Hoffman and A. Rahman, *J. Chromatogr.*, 473 (1989) 260.
9 N.E. Hoffman and J.H.Y. Chang, *J. Liq. Chromatogr.*, 14 (1991) 651.
10 S.K. MacLeod, D.T. Fagan and J.P. Scholl, *J. Chromatogr.*, 502 (1990) 236.
11 J.W. Dolan, *LC·GC*, 9 (1991) 22.
12 B.B. Sithole, B. Zvilichovsky, C. Lapointe and L.H. Allen, *J. Assoc. Off. Anal. Chem.*, 73 (1990) 322.
13 L. Simonson and K. Nelson, *LC·GC*, 10 (1992) 533.
14 A. Zlatkis and R.P.J. Ranatunga, *Anal. Chem.*, 62 (1990) 2471.