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Direct injection of human plasma samples after ultrafiltration into programmed temperature vaporiser-gas chromatography–mass spectrometry with packed liner

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Abstract

The direct injection of plasma samples after ultrafiltration into a gas chromatograph using a packed injector liner was investigated. Ropivacaine, a local anaesthetic of the amide type and one of its metabolites (PPX) were used as model compounds in this evaluation. Phosphoric acid was added to the plasma to minimize the protein binding. After ultrafiltration, 50µl of the sample was directly injected into the chromatographic system. No interfering peaks or damage to the GC or MS system were observed using ultrafiltration as a sample-preparation method. The validation of the method demonstrated good linearity and selectivity .The limits of quantification were 1.1 n*M* (301 pg/ ml) and 1.4 n*M* (325 pg/ ml) for ropivacaine and PPX, respectively. The liner had to be changed after 20 injections. @ 2001 Elsevier Science B.V. All rights reserved.

Keywords: Direct injection; Ropivacaine

1. Introduction

The separation of pharmaceuticals by capillary gas chromatography (CGC) has evolved considerably over the last decade through the introduction of new and selective stationary phases. Also, the more recent deactivated fusedsilica columns now offer versatile chromatographic performance for many compounds in this area. The large volume injector (LVI) or programmed temperature vaporiser (PTV) as an injecotr for CGC has been developed to improve

the detection limits of analytical methods. In addition, the PTV is used for the coupling of CGC to the sample-preparation methods such as liquid-liquid or solid-phase extraction [1-6]. The use of the PTV with packed liner for the injection of plasma after solid-phase extraction into CGC has been reported [7].

The aim of the sample-preparation method was to remove interferences from the biological sample, and it should be also reproducible with a high recovery, involving a minimum number of working steps. For plasma samples, different types of sample-preparation were tested. It was difficult to obtain high recovery for the metabolites using liquid-liquid extraction

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[8]. Solid-phase extraction (SPE) gave both high recovery and satisfactory chromatography, but took more time and more steps compared to ultrafiltration [9,10]. Ropivacaine is a new amide-type local anaesthetic drug, mainly used for surgery and for post operative pain relief. It also has lower central nervous and cardiotoxic potential than bupi-vacaine [II].

The aim of this study was to investigate the possibility of injecting biological fluids directly into a GC system after ultrafiltration using a packed injection liner. Ropivacaine and one of its metabolites (PPX) were used as model compounds to develop a rapid and simple GC method. Direct injections of plasma samples will be of substantial benefit in analytical issues, e.g. in connection with metabolic profiling and quantitative determination in bioanalysis.

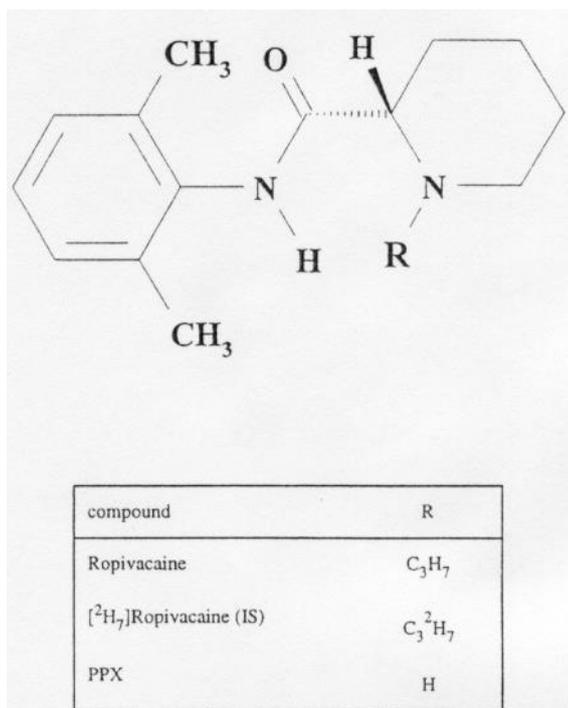| compound	R
Ropivacaine	C_3H_7
[$^2H_7$]Ropivacaine (IS)	$C_3{}^2H_7$
PPX	H

Fig. 1. The structure of ropivacaine and its metabolite PPX.

2. Experimental

2.1. Reagents and chemicals

Ropivacaine and its metabolite, in hydrochloride form, and the internal standard $^2H_7$-ropivacaine (Fig. 1) were supplied by the Department of Medicinal Chemistry, AstraZeneca (Södertälje, Sweden).

2.2. Apparatus.

The gas chromatographic analysis was performed using a Hewlett-Packard model HP 5890 Series II equipped with a PTV The PTV system was an OPTIC 2 (ATAS International, Veldhoven, The Netherlands). The liners used were 80mmX3.2mm and packed with ATAS "A" packing (a modified Chromosorb-based material that had been specially treated). The PTV conditions are: vent flow 100 ml/min, vent time 7 min (evaporation time), purge flow 2 ml/min (purge pressure 5 p.s.i.), split flow 50 ml/min and split open time 2 min. The injector temperature was set at 50°C and after the evaporation period, the temperature was raised by 5°C/s to 300°C. Helium was used as carrier gas and was obtained from AGA (Lidingö, Sweden). The oven temperature was programmed for an initial hold of 2 min at 80°C, and then an

increase of 30°C/min until a temperature of 330°C was reached. The column was BPX35 (35% phenyl-(equiv.) polysilphenylene-siloxane) and was purchased from SGE (TX, USA). The mass spectrometric instrument was a Finnigan SSQ 710B (San Jose, CA, USA). Conditions for MS measurements were: MS transfer line at 330°C, ion source at 250°C, electron impact ionization at 70 eV and scan mode 70-300 amu (5 scans/s). The ions corresponding to ropivacaine, PPX and I.S. are 126, 84 and 133 amu, respectively.

A Centrisart I ultrafiltration kit (20 000 cut-off) obtained from Sartorius (Goettingen, Germany) was used for ultrafiltration of the plasma samples.

2.3. Sample preparation

2.3.1. Ultrafiltration
The plasma sample was thawed and carefully homogenized using a whirl mixer. A volume of 500µl plasma sample was mixed with 75µl of 2 M phosphoric acid in a Centrisart I

ultrafiltration kit (20 000 cut-off). A 50-µl sample of internal standard solution was added and then centrifuged at 3500 rpm at 25°C for 20 min. The ultrafiltrate was directly injected (50µl) into the chromatographic system.

2.4. *Validation*

Each calibration curve consisted of six to seven calibration points covering the range from 1.14 to 1140 nmol/l for ropivacaine and from 1.49 to 1490 nmol/l for PPX. The peak area ratios for ropivacaine, PPX and the internal standard (ropivacaine-D7) were measured and a standard curve without the zero concentration was constructed.

3. Results and discussion

3.1. *Method development*

The aim of the present work was to study the direct injection of ultrafiltrate of plasma samples into a GC-MS using a PTV system. Ropivacaine and one of its metabolites (PPX) in plasma samples were used. A simple sample-preparation, shortseparation time and a low quantification limit were the aim in this study.

A most important part of bioanalysis is samplepreparation. The aim of the sample-preparation method was to remove interferences from the biological sample, and the method should also be reproducible with a high recovery, involving a minimum number of working steps. Direct injection of biological fluids into chromatographic systems can simplify the method as well as shorten the analysis time. In this study the direct injection of plasma after ultrafiltration was investigated. Phosphoric acid was added to the plasma to minimize the protein binding. After ultrafiltration the sample was directly injected into the chromatographic system. No interfering peaks and no effect on GC or MS system was observed using ultrafiltration as a sample-preparation method. PTV linearity is tested and the method is validated.

3.2. *PTV linearity*

According to the manufacturer, the liner liquid capacity is 150µl using organic solvents. In our study plasma water was injected, for which reason the relationship between relative response and injection volumes (10- 250µl) was examined. The PTV system was found to give a linear response up to 100µl (Fig. 2).

3.3. *Validation*

3.3.1. *Selectivity and linearity*

The validation procedure carried out used six to seven standards, three QC samples at three different concentrations and blank (calibration points were measured in duplicate).

Every run was carried out with the same liner. Plasma spiked with a mixture of ropivacaine, metabolite and the internal standard was analyzed and compared to blank plasma samples. No interfering compounds were detected. Fig. 3 shows that the method is selective. The peak in the mass chromatogram of *m/e* 126 at Fig. 3 (blank plasma) is a memory effect from a plasma sample that was injected before the blank.

The results showed a linear response for all analytes in the calibration range studied (Fig. 4). The linear regression correlation coefficients (R^2) were 0.9991, 0.9997 and 0.9969 (intercept of (Y): 0.08, -0.002 and 0.003, slope: 0.025, 0.03 and 0.027, P<0.0001) for PPX. For ropivacaine the R^2 values were 0.9998, 0.9974 and 0.9969 (intercept of (Y): -0.001, 0.02
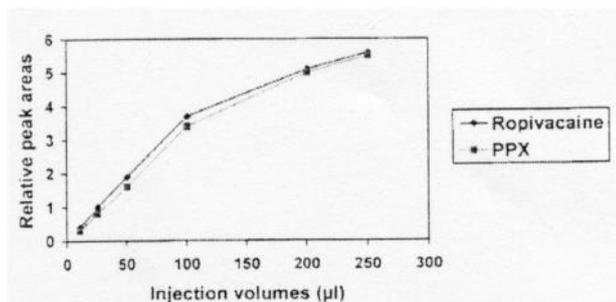

Fig. 2. Relationship between ultrafiltrate plasma injected volume and relative peak areas.
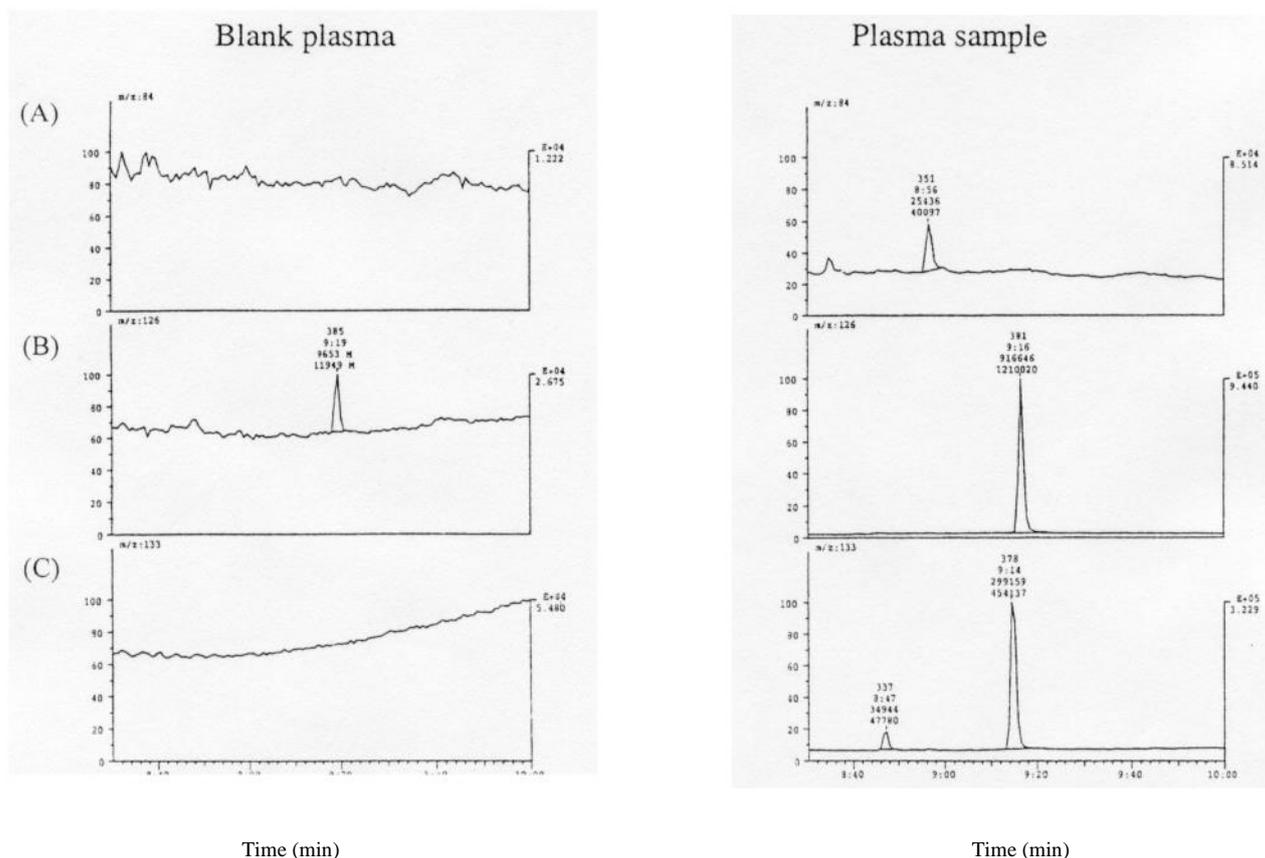
Fig.3. Chromatograms obtained from extracted blank plasma and from blank plasma spiked with analytes (400 nM each) and I.S.: (A) PPX *(m/z:* 84), (B) ropivacaine *(m/z:* 126) and (C) internal standard ropivacaine-D7 *(m/z:* 133).

and 0.06, slope: 0.06, 0.08 and 0.08, P<0.0001) using ultrafiltrate.

3.3.2. *Accuracy and precision*

The accuracy is determined as the ratio of the found and the theoretical concentration for three concentrations (12.5, 418 and 835.9 n*M, n*=4) of ropivacaine, while for PPX the concentrations were 11.2, 380.9 and 761.7 n*M (n*=4). The recoveries ranged from 102 to 112% for ropivacaine and from 110 to 117% for PPX (Table 1).

The precision is determined by the percentage coefficient variation of the within- and between-day variations at three different concentrations (12.5,418 and 835.9 n*M)* of ropivacaine, while for PPX the concentrations were 11.2, 380.9 and 761.7 n*M.* The precision was consistently -3-12% for within-day measurements.

The data of between-day variation of the precision were in the range 8-14% for ropivacaine and 8-16.1% for PPX. The precision results are summarized in Table 1.

3.3.3. *Liner long-term use*

The liner (AT AS " A ") was tested for different numbers of injections (50μl). It could be used for ~20 injections. The MS response decreased after 15 injections and disappeared totally after 20 injections. The colour of the packing material changed from white to brown and eventually became dark brown. When a new liner was installed, a good response was obtained again. This may be due to the plasma proteins that adsorbed to the liner packing (proteins with molecular weight lower than 20 000 amu will pass through the ultrafiltration filters).
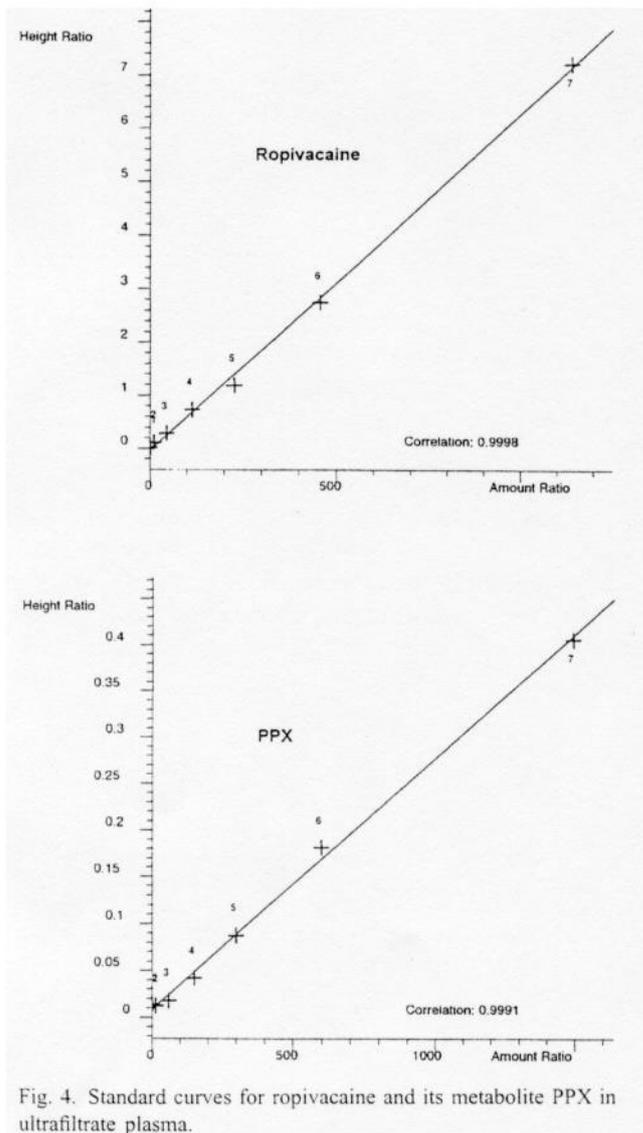
Fig. 4. Standard curves for ropivacaine and its metabolite PPX in ultrafiltrate plasma.

3.3.4. *Carry-over*

The carry-over was tested by injecting blank after the highest standard concentration. The observed carry-over was less than 2% of the injected sample.

4. Conclusions

It has been demonstrated that it is possible to inject ultrafiltrate plasma directly into a GC system using a PTV with packed injector liner. The linearity was satisfactory in the calibration range studied for all compounds. The results demonstrate that the method is accurate. One limitation is that the liner must be changed after 20 injections. It may be possible to obtain a longer lifetime for the liner by using a back-flash system for the PTV or by injecting less plasma volumes (10-20μl). Furthermore, due to the matrix components, a selective detector such as mass spectrometry (MS) is necessary.

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Table 1
The accuracy and precision (within- and between-day) at various concentrations in plasma

Analyte	Concentration (nM)	Accuracy (n = 8) (%)	Precision	
			Intra-day (n = 4) (RSD%)	Inter-day (n = 3 days) (RSD%)
Ropivacaine	12.5	102	12	14
	418	104	8	7
	835.9	112	3	8
PPX	11.4	117	7	8
	380.9	110	6	6
	761.7	111	8	12

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