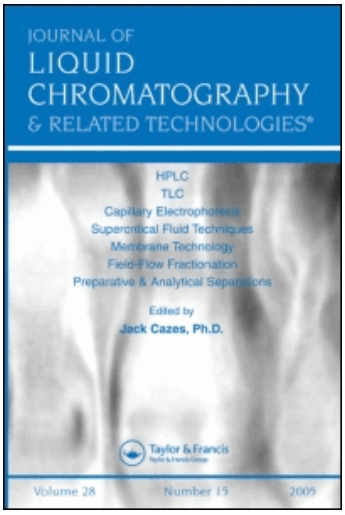


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Solid-Phase Extraction of Perphenazine from Blood Serum for High Performance Liquid Chromatographic Analysis

A. Hrdlička[a]; Z. Ďurd'ová[a]; E. Hadašová[ab]
[a] Department of Analytical Chemistry, Masaryk University Kotlářská, Brno, Czech Republic [b] Department of Pharmacology, Masaryk University, Brno, Czech Republic

**To cite this Article** Hrdlička, A. , Ďurd'ová, Z. and Hadašová, E.(1997) 'Solid-Phase Extraction of Perphenazine from Blood Serum for High Performance Liquid Chromatographic Analysis', Journal of Liquid Chromatography & Related Technologies, 20: 6, 935 — 942
**To link to this Article: DOI:** 10.1080/10826079708013664
**URL:** http://dx.doi.org/10.1080/10826079708013664

J. LIQ. CHROM. & REL. TECHNOL., 20(6), 935-942 (1997)

# SOLID-PHASE EXTRACTION OF PERPHENAZINE FROM BLOOD SERUM FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS

A. Hrdlička,† Z. Ďurd'ová, E. Hadašová‡

Department of Analytical Chemistry
Masaryk University
Kotlářská 2
611 37 Brno, Czech Republic



## ABSTRACT

A solid-phase extraction (SPE) process has been developed with the aim of replacing multistage liquid-liquid extraction that is currently used for sample clean-up before the chromatographic determination of perphenazine (PPZ) in serum.

The method comprises the trapping of PPZ from 2 mL of serum by 100 mg of silica gel with polymeric $C_{18}$ phase, followed by rinsing of the endogenous compounds with water and acetonitrile. The drug is eluted with 0.7 mL of methanol and the eluate is evaporated to dryness under stream of air. The residue is dissolved in 100 μL of methanol and 20-μL aliquot is injected onto an HPLC column packed with silica gel modified with cyanopropyl groups. To control the retention and separation of PPZ from the other compounds, ammonium acetate concentration in the mobile phase consisting of 90% v/v methanol has been varied from 10 to 40 mmol/L.

The SPE method enabled 86.2+5.0% of PPZ to be recovered which is 12% to 22% more than by liquid-liquid extraction procedure. The repeatability of 5.7% (rel.) and the limit of detection (S/N=3) of 1.3 ng/mL have been attained. The method has been verified on serum samples from patients medicated with PPZ.

## INTRODUCTION

The currently used neuroleptics show undesirable side effects if their doses are too high. However, their clinical efficacy seems to be related to sufficiently high plasma concentrations. Optimal therapeutic range may differ for each patient and, besides, varies in the course of the disease.[1] Plasma monitoring of the antipsychotic drugs is required for their more effective and safer use.



Perphenazine, 4-[3-(2-chloro-phenothiazine-10-yl)propyl]-1-piperazine-ethanol, (PPZ) belongs to the most potent phenothiazine antipsychotic compounds. These drugs are typically analyzed by chromatographic methods, i.e., by HPLC or, to a lesser extent, by GC.

At present, separation of fourteen, mostly phenothiazine drugs by packed (cyanopropyl) column supercritical fluid chromatography has been studied.[2] Sample clean-up prior to chromatographic determination of PPZ in blood serum is inevitable.

Perphenazine and its dealkylated metabolite were separated on an HPLC column packed with octadecylsilanized silica gel by using a mobile phase consisting of methanol, water, dichloromethane, and ammonia.[3] For the determination of PPZ in serum, a cyanopropyl column and the mobile phase containing 90% v/v of methanol and 10% v/v of 10 mmol/l ammonium acetate were employed.[4]

Sample clean-up was performed by multistep liquid-liquid extraction, a cumbersome and time consuming process with recoveries of 70%,[3] or 64.3+6.8 to 74.3+5.7%.[4]

In this work, a method for simple and rapid analysis of PPZ in human plasma is presented. The method consists in a solid phase extraction of PPZ followed by an HPLC determination with UV detection.

## EXPERIMENTAL

### Reagents and Solutions

Stock solution of perphenazine (a pharmaceutical preparation with minimum content of 98.5% PPZ, Léčiva Prague, Czech Republic), 1 mg/mL, and its working solutions (0.1 and 0.01 mg/mL) were prepared in methanol and stored under refrigeration.

Aqueous solution of EXAPAT, a stabilized lyophilized human serum (Imuna Šarišské Michalany, Slovakia) was used in SPE method development. Lyophilized serum and its solutions were stored under refrigeration. The solutions were not older than 10 days. Patient sera were stored in a freezer not longer than 12 days. Two mL of the EXAPAT solution (as a blank or spiked with PPZ) or patient serum were applied onto SPE cartridge as a sample.



Isothermally distilled ammonia, redistilled acetonitrile, and triethylamine distilled under reduced pressure were used. All other reagents were of analytical grade (Lachema, Czech Republic).

HPLC mobile phases were prepared in a volume flask by diluting the volume of an aqueous solution of ammonium acetate with methanol. To prepare the solution of ammonium acetate, 0.1 mol/L acetic acid was neutralized with ammonia to pH 7.25 and diluted with water.

### Apparatus

The HPLC equipment comprised of a model 8500 syringe pump (Varian, USA), a model RH 7125 injection valve (Rheodyne, USA) with 5-, 10- or 20-μL sample loop, a multiple wavelength detector, HP 1050, equipped with 1-μL cell (Hewlett-Packard, Japan) and a TZ 4520 recorder (Laboratory Instruments Prague, Czech Republic). CGC glass columns 150x3 mm i.d. and 30x3 mm i.d., packed with Separon SGX CN silica gel with cyanopropyl groups with particle size of 5 and 7μm, respectively, were used as the analytical column and guard column (all Tessek Prague, Czech Republic). The mobile phase was delivered at the flow rate of 0.5 mL/min.

The absorption curve of PPZ solution in 90% v/v methanol possessed maxima at 203 nm and 257 nm, the latter with slightly lower (91% rel.) absorbance value. HPLC detection of PPZ was performed at 257 nm.

Acidity was measured with a model OP-208 pH-meter equipped with an OP-0808-P glass-Ag/AgCI combined electrode adjusted by means of standard buffers of pH 2.1 and 7.0 (Radelkis, Hungary).

Silica-cart cartridges (Tessek Prague, Czech Republic) packed with 0.6 g of 60-μm oktadecylsilanized spherical silica gel and 1-mL Bakerbond-spe columns (Baker, USA) dry repacked with 100 mg of the sorbent were used in SPE method development. A Separon SGX RPS sorbent of polymeric character with the carbon content of 24% was utilized except some preliminary experiments where the Silica-cart cartridges were packed with Separon SGX $C_{18}$ with the carbon content of 18% (Tessek Prague, Czech Republic). The Silica-cart cartridges were processed by means of a syringe, the Bakerbond-spe columns by using a model Baker spe-12G System vacuum manifold (Baker, USA), both at the flow rate of 1 mL/min.



## RESULTS AND DISCUSSION

### HPLC Determination

The concentration of PPZ was determined by a modification of previously published HPLC method.[4] The HPLC column was packed with silica gel with bonded cyanopropyl phase. Due to the basic character of PPZ molecules, their interactions with residual silanols played an important role in the retention mechanism of PPZ. As a consequence, the variation of ammonium acetate concentration in the mobile phase between 10 and 40 mmol/L enabled the retention of PPZ and, also, its separation from the other compounds to be optimized.

The effect of ammonium acetate concentration on PPZ retention is illustrated in Fig. 1. Because of a low solubility of PPZ in aqueous media, the content of methanol in the mobile phase was kept constant at 90% v/v.

To determine the concentration of PPZ in SPE eluents, calibration curves based on peak heights were used. The curves measured for five standard solutions, four injections of each, were linear in the range from 0,001 to 10 μg/mL PPZ with correlation coefficient ranging typically from 0,9997 to 0,9999 and with a negligible value of y-intercept. In the analysis of a patient sera, PPZ standard was injected before and after the sample and the results were evaluated by external standard method.

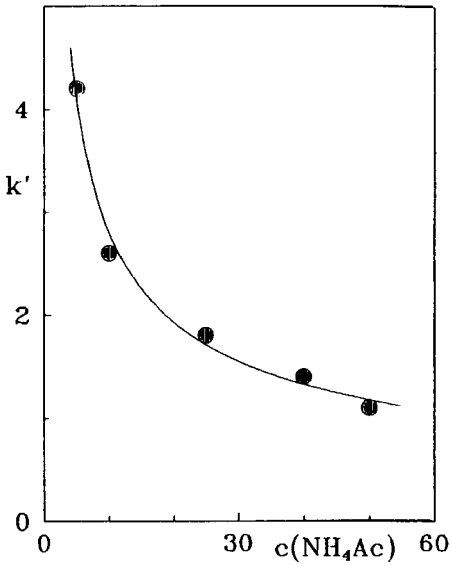


**Figure** 1. Perphenazine capacity factor as a function of ammonium acetate concentration, mmol/L, in the mobile phase. Column 150x3 mm i.d. and guard column 30x3 mm i.d., packed with 5-μm and 7-μm silica gel with cyanopropyl groups. Mobile phase: ammonium acetate in 90% v/v methanol, flow 0.5 mL/min. Samples: 5 μL of 0.4 μg/mL perhenazine in methanol.



## SPE Method Development

Silica-cart cartridges were conditioned with 2 mL of methanol and, consecutively, with 1 mL of water. Two mL of standard serum as a blank or spiked with PPZ were passed through the cartridge and this was rinsed with 2 mL of water and, in some experiments, also with 2 mL of acetonitrile. In the next step, elution of matrix components or that of PPZ were studied by using various solvents or solutions. PPZ elution profile was generated by passing 1-mL volumes of the solvent through a single cartridge. To evaluate the elution of the sample matrix, chromatograms of blank samples were compared mutually.

The application of the cartridges packed with Separon SGX $C_{18}$ was unsuccessful because of high retention of PPZ and band broadening. Good results were attained when the sorbent was replaced by Separon SGX RPS with polymeric $C_{18}$ phase and higher carbon content and, thus, with lower concentration of residual silanols that could interact with PPZ molecule. After

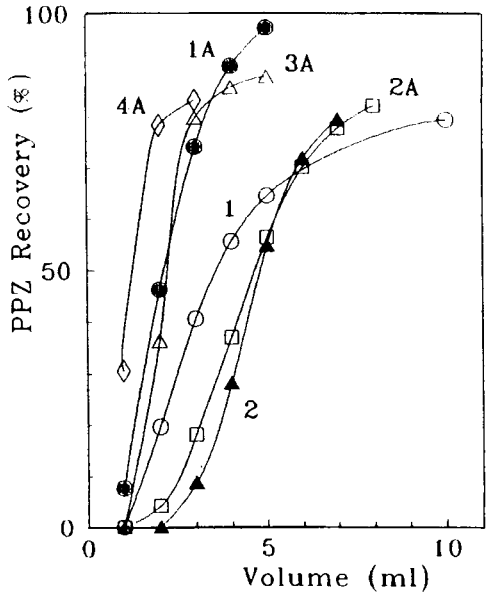


**Figure 2**. Elution profiles of perhenazine. Silica-cart cartridges packed with 0.6 g of Separon SGX RPS. Conditioning: 2 mL of methanol, 1 mL of water. Sample application: 2 mL of standard serum spiked with 1 μg/mL PPZ. Rinsing: 2 mL of water and, at the curves indicated with A, also 2 mL of acetonitrile. Elution: methanol (curves 1 and 1A); 1 mmol/L triethylamine in 90% v/v methanol (2, 2A); 10 mmol/L triethylamine in 90% v/v methanol (3A) and in 100% methanol (4A).



rinsing the cartridge with water, the matrix components were efficiently washed out with 2 mL of acetonitrile. It was verified that PPZ remains retained even when the cartridge is rinsed with 15 mL of acetonitrile. After rinsing step with acetonitrile, methanol was found as the most effective PPZ eluent as can be shown on the curves of cumulative % recovery vs. solvent volume in Fig. 2.

Aiming at the reduction of the excessive amount of sorbent used in the Silica-cart cartridges, these were replaced by 1-mL Bakerbond-spe columns repacked with 100 mg of Separon SGX RPS. The solid phase was conditioned with 1 mL of methanol and 0.5 mL of water. Endogenous compounds were washed out with 1 mL of water and 1 mL of acetonitrile after sample application. At these conditions, 2 mL of the standard serum spiked with 0.2 μg/mL PPZ was applied onto the SPE column and the drug recovery was estimated after elution with methanol, three measurements at each volume.

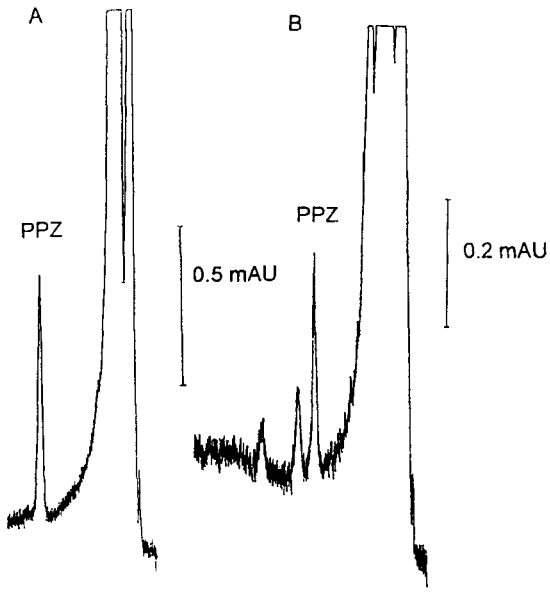


**Figure 3**. Chromatograms of standard serum spiked with PPZ and patient serum. Chromatogram A: 2 mL of standard human serum spiked with 10 ng/mL PPZ; concentration of ammonium acetate in the mobile phase 10 mmol/L; PPZ retention time 7.7 min. Chromatogram B: 2 mL of patient serum taken 4 hours after medication with a single dose of 16 mg of PPZ (0.35 mg/kg) and a daily dose of 25 mg of thioridazine. Other conditions as in A.



With 0.5 mL of methanol, 95.1% of PPZ was recovered. Further, practically 100% recovery was achieved by elution with 0.6 mL (99.3%), 0.7 (99.7%), and 0.8 mL (100.8%). The volume of 0.7 mL of methanol was found as sufficient to elute PPZ from the column. The recommended therapeutic range is 0.8 to 2.4 ng/mL PPZ in serum however, values as much as 12 ng/mL PPZ can be found because patients are frequently medicated with high doses of the drug.[5] In following SPE experiments, the PPZ concentration in serum was lowered to 10 ng/mL. The methanolic eluate was evaporated to dryness under stream of air and reconstituted with 100 μL of methanol. The volume of the solution injected onto HPLC column was increased to 20 μL, i.e. to a maximum sample volume that still did not cause band broadening and peak shape deformation. For five determinations of PPZ, the recovery of 86.2 ± 5.0% and the repeatability of 5.7%, expressed as relative standard deviation, were found. The observed decrease of the recovery from 99.7% to 86.2% is partly due to the preconcentration step (ca. 5% of the total difference of 13.5% as verified by

experiments with evaporation of 10 ng/mL PPZ methanolic solutions and dissolution of their residues), partly it could be explained by losses in the sample processing because these can have greater significance when working with PPZ concentrations in low nanogram range. The limit of detection, 1.3 ng/mL, based on a signal to noise ratio S/N=3 was comparable with the value of 0.75 ng/mL given in literature[4] and could be improved by using better quality HPLC column.

The method was verified on serum samples from patients medicated with perphenazine and, simultaneously, with some other pharmaceutics. No interferences caused by the presence of the other pharmaceutics were observed. In Fig. 3, chromatogram of standard serum sample spiked with PPZ and that of patient serum are introduced. The SPE method developed represents rapid and more efficient alternative to recently used liquid-liquid extraction of perphenazine from serum samples.



## REFERENCES

1. A. E. Balant-Gorgia, L. Balant, Clin. Pharmacokinetics, **13**, 65 (1987).

2. T. A. Berger, W. H. Wilson, J. Pharmac. Sciences, **83**, 281 (1994).

3. N. E. Larsen, L. B. Hansen, P. Knudsen, J. Chromatogr., **341**, 244 (1985).

4. M. C. Dessalles, B. Vienne, G. Mahuzier, J. Chromatogr., **487**, 215 (1989).

5. F. Haffner, "Perphenazine Serum Levels in Patients on Standard Doses", in **Clinical Pharmacology in Psychiatry**, S. G. Dahl and L. F. Gram, eds., Pharmacology Series 7, Springer Verlag, Berlin, 1989, p.281.

†Author for correspondence. Present address: Department of Environmental Studies, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic.

‡Department of Pharmacology, Masaryk University, Joštova 10, 662 43 Brno, Czech Republic.

Received July 22, 1996
Accepted August 27, 1996
Manuscript 4243