Anexa 1.

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Cent. Eur. J. Chem. • 10(4) • 2012 • 1380-1388
DOI: 10.2478/s11532-012-0058-5

Central European Journal of **Chemistry**

Use of an immiscible diluent in ionic liquid / ion pair LC for the assay of an injectable analgesic

Research Article

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Received 8 February 2012; Accepted 21 April 2012

Abstract: Injectable solutions used in treatment of intense pain are based on combinations of active ingredients such as metamizole sodium (MTZ), pitofenone hydrochloride (PTF) and fenpiverine bromide (FPB). The simultaneous chromatographic assay of such combinations poses difficulties due to their structural variety, highly polar character, and wide concentration ranges (500 mg mL^{-1} for MTZ, 2 mg mL^{-1} for PTF and 0.02 mg mL^{-1} for FPB). Fast hydrolysis of MTZ on aqueous dilution causes additional problems due to impurity (MTC) formation. Sodium hexane sulphonate (10 mM) was used as ion pairing agent for PTF, FPB and MTC in a mobile phase consisting of 48/52 (v/v) methanol and aqueous 0.2% triethylamine at pH=3. The ionic liquid 1-butyl-1-methyl-pyrrolidinium tetrafluoroborate (10 mM) was used as mobile phase additive to preserve the MTZ peak symmetry. The minor active ingredient FPB was selectively extracted into 1-octanol by ion pair formation with picric acid. A 20 μL aliquot of the organic layer was directly injected into the column.

Keywords: *Ionic liquids addition • Ion-pair separation mechanism • Injection of immiscible diluent • Analgesic combination.*

1. Introduction

High throughput in modern drug quality control requires assay of as many analytes as possible, in the shortest time, in a single analysis. When targeting structurally related compounds a single separation mechanism is usually considered using common selectivity enhancement techniques. When the compounds in a pharmaceutical combination exhibit a variety of structural characteristics (*i.e.*, analytes containing opposite permanent charges or functional groups forming oppositely charged moieties upon dissociation), their separation is likely to be more complicated. The range of separation mechanisms can be extended by addition of buffers, ion-pairing agents or ionic liquids [1,2]. If the compounds are present in very different amounts, selective isolation may be employed. Injectable solutions used for the symptomatic treatment of intense pain accompanied by smooth muscle spasms are often based on combinations of metamizole sodium (MTZ), fenpiverine bromide (FPB) and pitofenone hydrochloride (PTF), which produce synergistic rapid analgesic and spasmolytic action.

Due to its extensive and long term use, numerous methods have been reported for analysis of MTZ alone or in combination with other drugs in pharmaceutical formulations. These techniques include flow injection spectrophotometry [3], capillary electrophoresis (CE) [4], UV spectrophotometry [5-8], chemiluminescence [9] and liquid chromatography [10-12]. However, little literature was found on the simultaneous determination of MTZ, FPB and PTF. Ratio-spectra zero-crossing 1st order derivative spectrophotometry [8,13] was applied to determine the three drugs in injectable solutions. Liquid-liquid extraction into chloroform followed by UV-Vis spectrometric determination [14] was used to assay only FPB in a tablet also containing MTZ and PTF. Thin layer chromatography (TLC) with direct densitometric detection was used to assay MTZ, PTF and FPB among other pharmaceuticals like codeine and etenzamide in sugar-coated tablets [15]. Fluorescent silica gel plates were eluted with benzene – methanol – diethylamine (3:3:1) and detection at 254 nm. No liquid chromatographic methods for simultaneous determination of MTZ, FPB and PTF were found in the literature.

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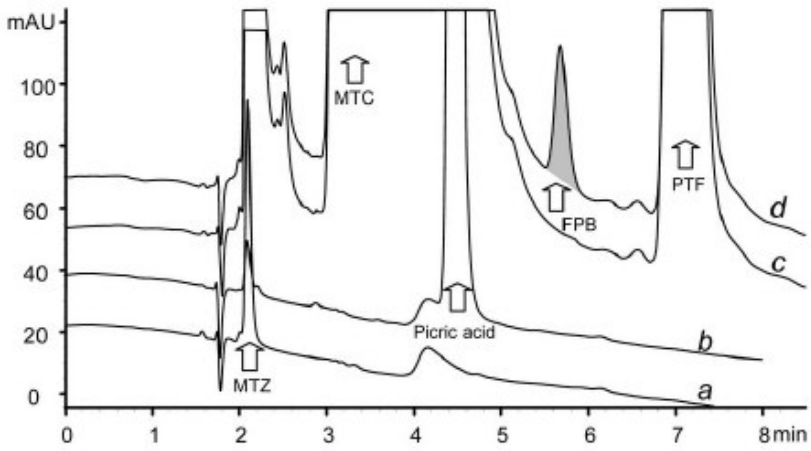

Figure 5. Selectivity of the FPB liquid-liquid extraction procedure: a) extraction of MTZ alone into 1-octanol; b) extraction of MTZ alone into 1-octanol, after addition of the ion pairing agent (picric acid) to the aqueous phase; c) extraction of MTZ, MTC and PTF into 1-octanol using picric acid as ion pairing agent; d) extraction of the sample (analgesic injectable solution) as described in section 2.4.

and PTF, (c) standard, and (d) sample were injected. Illustration of the method's selectivity is given in Fig. 5. Blank samples showed no interferences with the FPB peak and in chromatograms obtained after injection of the standard FPB solution no interfering peaks were observed.

Resolution between picric acid/FPB and FPB/PTF peak pairs in the standard FPB solution chromatogram was 5.3 and 3.9, respectively. RSD% for intra- and inter-day repeatability (n = 6) was below 2% with bias 0.9 – 1.6%. Accuracy was evaluated at 90, 100 and 110% of FPB nominal concentration and provided recoveries between 100.9 – 102.6% and RSD% values between 0.4 – 1.8%. Sample stability was assessed on extracted samples of FPB both at room temperature (25°C) and cold conditions (light protected at 6°C). Samples were stable (maximum 2% peak area decrease) for 1 day at room temperature and 2 days at 6°C.

4. Conclusions

A rapid, sensitive, selective and accurate ionic liquid/ion-pair RP-LC method was developed for the simultaneous assay of MTZ, FPB, PTF and MTC in an injectable analgesic solution. Ion pairing based liquid-liquid extraction into 1-octanol was used to isolate FPB from MTZ, increasing sensitivity and selectivity. FPB was extracted into 1-octanol as an ion pair with picric acid and the organic layer was directly injected onto the column. Direct injection caused no negative effects on peak shapes or efficiency and avoids tedious and error-prone sampling, solvent evaporation and residue re-dissolution. The liquid-liquid extraction procedure required 16 minutes and provided good accuracy.

The method was validated and applied to the assay of all four compounds in an injectable pharmaceutical on the Romanian market. This demonstrates the usefulness of large volume injection of immiscible diluents in RP-LC applications.

Acknowledgements

Toma Galaon aknowledges support by the strategic grant POSDRU/89/1.5/S/58852, *Postdoctoral programme for training scientific researchers* cofinanced by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007-2013. Andrei Medvedovici acknowledges support by the Romanian project PNII_ID_PCE_2011_3_0152.