Journal of Chromatography, 456 (1988) 45–51
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 858

FULLY AUTOMATED ANALYTICAL SYSTEM USING LIQUID–SOLID EXTRACTION AND LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF CGP 6140 IN PLASMA

M. C. ROUAN*, J. CAMPESTRINI, J. B. LECAILLON and J. P. DUBOIS
Laboratoires Ciba-Geigy, Biopharmaceutical Research Centre, B.P. 308, 92506 Rueil-Malmaison Cedex (France)
and
M. LAMONTAGNE and B. PICHON
Gilson Medical Electronics S.A., B.P. 45, 95400 Villiers-le-Bel (France)

SUMMARY

Liquid–solid extraction on disposable extraction columns (DECs) and liquid chromatography can be combined in a completely automated analyser. The Gilson ASPEC system was used to develop a procedure for the determination of CGP 6140 in plasma. Both sample preparation via C_8 Bond-Elut DECs and injection were fully automatic.

The fully automated system prepared the samples by performing the same operations as for a manual procedure. The DEC was first wetted with methanol, then with water. A 400-μl volume of plasma and 40 μl of the internal standard solution, diluted with 1 ml of water, were applied to the DEC, rinsed with 10^{-2} mol/l dipotassium hydrogenphosphate and eluted from the DEC with 300 μl of acetonitrile–methanol (50:50, v/v). The eluting strength of the eluate was reduced by dispensing 1 ml of water into each vial prior to direct injection into a Spherisorb ODS column via a 1-ml loop. This allowed the reconcentration of the extracted compounds on the top of the column, as they were injected in a large volume of solvent of lower eluting strength than the mobile phase [acetonitrile–methanol–$4 \cdot 10^{-3}$ mol/l ammonia solution (54.5:5:40.5, v/v/v)]. Reproducibility results are presented.

INTRODUCTION

Liquid–solid extraction (LSE) via disposable extraction columns (DECs)[1] or by column switching[2,3] is being increasingly used in combination with high-performance liquid chromatography (HPLC) as an alternative to time-consuming liquid–liquid extraction[4]. Semi- or fully automated systems for LSE and HPLC have recently been introduced. The advanced automated sample processor (AASP) allows semi-automatic extraction on a DEC and on-line elution into the analytical column[5]. The Zymate Laboratory Automation System permits full automation of LSE and HPLC[6], but the high cost of this instrument limits its application. The PROSPEKT

0021-9673/88/$03.50 © 1988 Elsevier Science Publishers B.V.

system, still under development, should permit on-line liquid–solid extraction and on-line elution into the analytical column[7].

This paper describes a fully automated procedure for the determination in plasma of a compound with antiparasitic properties, CGP 6140. Both sample preparation via C_8 Bond-Elut DECs and injection were fully automated using the Gilson ASPEC system. The procedure described is a modification of a method previously reported using the AASP system for sample preparation[8].

EXPERIMENTAL

Materials and reagents

CGP 6140 [4-nitro-4′-(N-methylpiperazinylthiocarbonylamido)diphenylamine] and CGP 10 631 (4-nitro-4′-acetylaminodiphenylamine) (internal standard) were provided by Ciba-Geigy (Basle, Switzerland). Their structures are shown in Fig. 1. Internal standard and calibration solutions were prepared by dissolving the compounds in methanol and by further dilution with water–methanol (80:20, v/v).

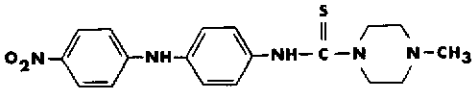
Dipotassium hydrogenphosphate and 25% ammonia solution were purchased from E. Merck (Darmstadt, F.R.G.). Acetonitrile was of HPLC quality (Carlo Erba France, Puteaux, France) and methanol was of RPE-ACS quality (Carlo Erba France).

Apparatus

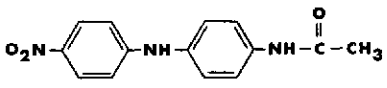
The chromatographic system consisted of a Model 303 pump (Gilson, Villiers-le-Bel, France), an ASPEC (Automatic Sample Preparation with Extraction Columns) system (Gilson) and a Model 773 UV detector (Kratos, Paris, France) set at 405 nm. A Model 4100 computing integrator (Spectra-Physics, Les Ulis, France) was used for data acquisition.

The ASPEC system combines three components (Fig. 2): an automatic sampling injector module, a Model 401 dilutor/pipettor and a set of racks and accessories, necessary for handling DECs and solvents.

LSE has been totally automated as a result of the development of a specific rack. This rack consists of three parts: DEC holder, drain cuvette and collection rack (Fig. 3). The DEC holder is mobile, so that each DEC can be automatically located



CGP 6140



CGP 10 631 (internal standard)

Fig. 1. Structures of CGP 6140 and of the internal standard CGP 10 631.

Fig. 2. Gilson ASPEC system.

either above the drain cuvette (Fig. 3A) or above a collection tube (Fig. 3B), according to the extraction step being performed. After extraction, the collected fraction can be injected in a Rheodyne injection valve for on-line HPLC analysis (Fig. 3C). The solvents required during the extraction process can be aspirated from four different bottles located on a solvent bottle rack.

With the ASPEC, the flow of the solvents through the DEC is carried out under a positive pressure. Precise volumes are delivered by the 401 dilutor, and forced

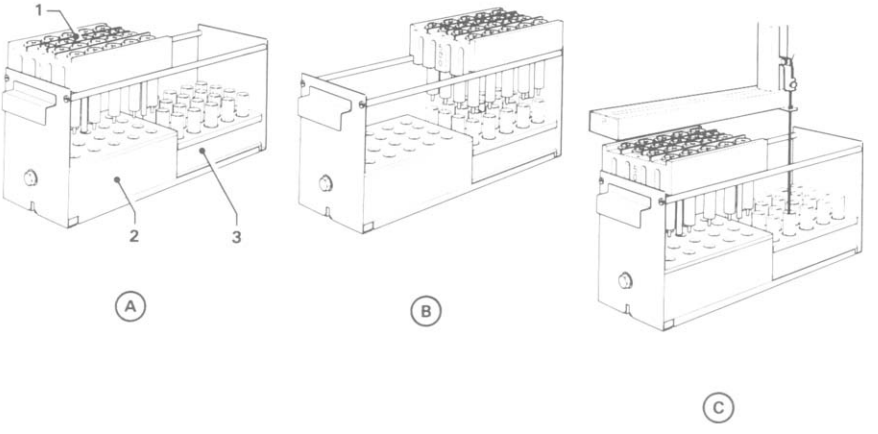


Fig. 3. Liquid–solid extraction rack of the Gilson ASPEC system. 1, DEC holder; 2, drain cuvette; 3, collection rack.

through the packing by air. A special cap fitted on each DEC assures air-tightness when the needle is dispensing liquid or air.

Column

The analytical column was a stainless-steel tube (25 cm × 4.7 mm I.D.) packed with Spherisorb ODS 1, particle size 5 μm (SFCC, Gagny, France).

Disposable extraction columns

C_8 Bond-Elut DECs (100 mg) of capacity 1 ml were used. They were manufactured by Analytichem International (Harbor City, CA, U.S.A.) and supplied by Prolabo (Paris, France).

Sample preparation

A 400-μl volume of plasma, 1 ml of water and 40 μl of the internal standard or calibration solution were transferred manually into a vial and shaken on a Vortex mixer for a few seconds. The vial was then placed on the appropriate rack of the Gilson ASPEC system.

All of the following operations on the samples were performed automatically by the ASPEC system. The automatic sequences were:

(1) DEC conditioning. Draw up 2 ml of methanol from bottle 1 and dispense into the DEC.

Draw up 2 ml of water from bottle 2 and dispense into the DEC.

(2) Liquid solid extraction. Draw up 1 ml of dilute plasma sample from a tube and dispense into the DEC.

Draw up 2 ml of 10^{-2} mol/l dipotassium hydrogenphosphate solution from bottle 3 and dispense into the DEC.

Shift the rack containing the DECs on top of the part of the rack containing the collection tubes.

Draw up 300 μl of methanol–acetonitrile (50:50, v/v) from bottle 4 and dispense into the DEC. The eluate is collected in the tube positioned under the DEC.

Pull back the rack containing the DECs.

(3) Injection. Draw up 1 ml of water from bottle 2 and dispense into the tube containing the eluate.

Draw up 1 ml of air and dispense into the tube, the needle being lowered into the tube to mix the liquids by bubbling.

Draw up 1.2 ml of mixture from the tube and dispense through the 1-ml injection loop.

Inject into the analytical column.

Each plasma sample was prepared separately during the chromatography of the previous sample. In all instances the needle was rinsed with 2 ml of water and a 40-μl segment of air was created before pipetting the liquid to be transferred, in order to avoid cross-contamination.

Chromatography

The chromatography was carried out at ambient temperature. The mobile phase was acetonitrile–methanol–$4 \cdot 10^{-3}$ mol/l ammonia solution (54.5:5:40.5, v/v/v). The flow-rate of the mobile phase was 1.2 ml/min.

RESULTS AND DISCUSSION

Automatic procedure

The ASPEC system prepared plasma samples by performing the same operations as in a manual procedure: the DEC was first conditioned, dilute plasma was then applied to the DEC and washed with an aqueous solution of dipotassium hydrogenphosphate before elution of the retained compounds with an organic solvent.

CGP 6140 was strongly retained on the DEC sorbent and 300 μl of methanol–acetonitrile (50:50, v/v) were necessary to elute it from the DEC. Generally, in manual procedures combining LSE and HPLC, either the sample collected from the DEC is evaporated to dryness and the residue is dissolved in the mobile phase for injection, or an aliquot of eluate is directly injected on to the column. However, it has been shown that at least 1 ml of sample can be injected without peak broadening if the drug is dissolved in a solvent whose eluting strength is less than that of the mobile phase[9]. The sample is then sorbed on the bonded phase at the head of the column until the injection is finished. Therefore, in the present procedure, the sample collected from the DEC was diluted by the ASPEC system with 1 ml water. The proportions of acetonitrile and methanol in the injected sample were then 11.5% each, whereas they were 54.5 and 5%, respectively, in the mobile phase. When 1 ml of this dilute sample was injected, no peak broadening was observed compared with the injection of 100 μl of CGP 6140 and CGP 10 631 in water. Almost all of the CGP 6140 extracted from plasma could then be injected on to the analytical column, thereby affording good sensitivity.

C_2 Bond-Elut DECs of capacity 2.8 ml and containing 500 mg of sorbent were also used. A high volume (1.5 ml) of methanol–acetonitrile (50:50, v/v) was necessary to elute CGP 6140 and CGP 10 631 from the DEC. It would have been necessary to inject several millilitres of dilute sample on to the column to obtain good sensitivity. Therefore, as no improvement of the separation between CGP 6140 or CGP 10 631 and endogenous plasma compounds was observed with these 500-mg DECs, the smaller 100-mg DECs were preferred.

The time required for sample preparation and injection (10 min) was less than the duration of the chromatography (18 min). Therefore, no attempt was made to shorten the time of the robotic procedure, but this should be feasible by increasing the flow-rate of sampling in some of the automated operations.

Reproducibility and accuracy

Examples of chromatograms are given in Fig. 4. The ratio of the peak areas of CGP 6140 and CGP 10 631 (internal standard) was plotted against the CGP 6140 concentration in plasma. An example of a calibration graph is given in Fig. 5. The equation of the graph was calculated by the least-squares method using weighted linear regression with a weighting factor of $1/(\text{concentration})^2$ (ref. 10).

The within-day reproducibility and accuracy of the method were calculated by analysing, on the same day, replicate plasma samples spiked with different concentrations of CGP 6140 (Table I). The limit of quantitation was 54 nmol/l of plasma (about 20 ng/ml).

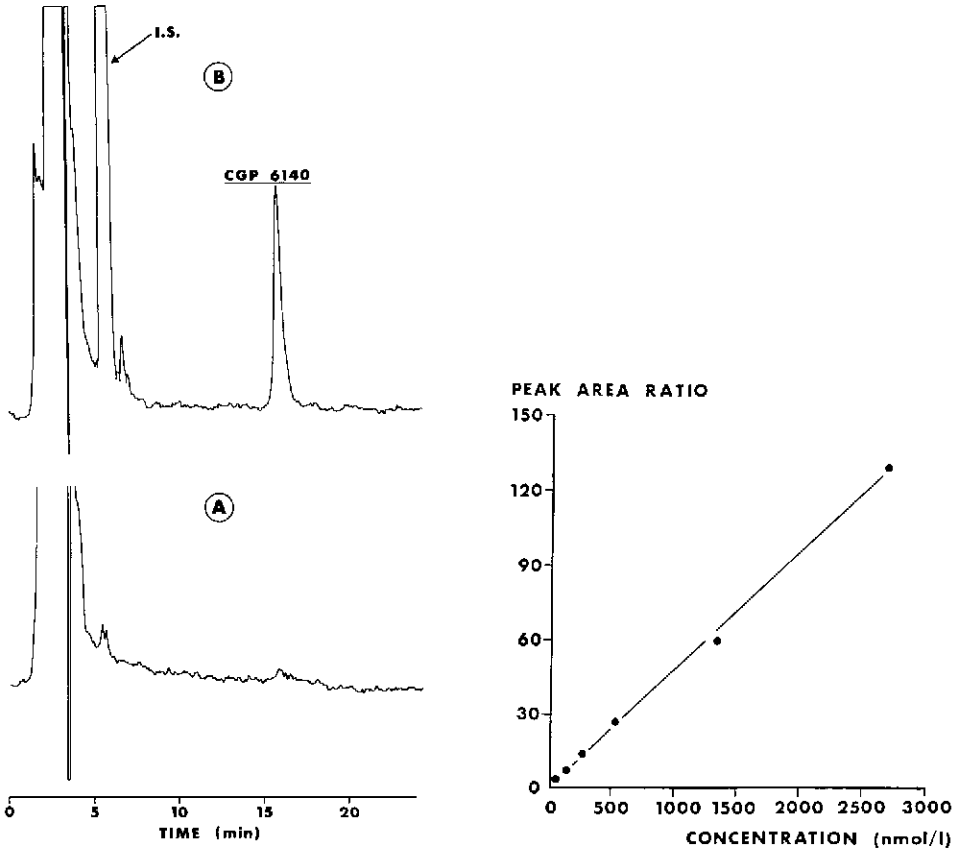


Fig. 4. Chromatograms of extracts of (A) blank plasma and (B) plasma containing 134.6 nmol/l of CGP 6140 and 5.92 μmol/l of CGP 10 631 (internal standard).

TABLE I

WITHIN-DAY ACCURACY AND REPRODUCIBILITY OF THE ASSAY OF CGP 6140 IN SPIKED PLASMA SAMPLES

To convert the results into ng/ml, multiply by 0.3715.

Introduced (nmol/l)	*No. of assays*	*Mean recovery ± S.D. (%)*	*Overall recovery ± S.D. (%)*
53.8	6	94 ± 9	
137	6	101 ± 9	
269	6	104 ± 8	100 ± 8
539	5	104 ± 5	
2693	5	98 ± 5	

Comparison with the AASP system

The procedure was compared with that previously reported for the determination of CGP 6140 in plasma using the AASP system for sample preparation[8]. With the ASPEC system, DECs are automatically and separately processed up to and including injection; elution is carried out at a constant flow-rate in each DEC. With the AASP system, ten DECs are first manually and simultaneously processed by elution at a constant pressure, then automatically eluted on to the column.

The reproducibility and the limit of quantitation of the procedure using the AASP and the Gilson ASPEC system for sample preparation were similar.

With the ASPEC system, CGP 6140 was stored in dilute plasma before injection and was stable for at least 10 h (mean recovery $\pm$ S.D. = 104 $\pm$ 8%, n = 6). Refrigerated racks can be installed on the ASPEC system, thereby increasing the sample throughput for this system. With the AASP system, CGP 6140 was stored in the DEC at ambient temperature before injection and slight degradation of the drug was observed after 8 h of storage.

CONCLUSION

Liquid–solid extraction on Bond-Elut DECs and HPLC can be combined in a completely automated analyser for the determination of CGP 6140 in plasma. The previously described method could be rapidly adapted. The major part of the CGP 6140 plasma extract could be injected on to the analytical column in a large volume of non-eluting solvent, thereby allowing good sensitivity.

REFERENCES

1 E. Doyle, J. C. Pearce, V. S. Picot and R. M. Lee, *J. Chromatogr.*, 411 (1987) 325.
2 H. P. Fiedler and H. P. Reuschenbach, *Chromatographia*, 19 (1984) 246.
3 D. Westerlund, *Chromatographia*, 24 (1987) 155.
4 M. W. F. Nielen, R. W. Frei and K. A. Th. Brinkman (Ch. 1), and R. Huber and K. Zech (Ch. 2), in R. W. Frei and K. Zech (Editors), *Selective Sample Handling and Detection in HPLC, Part A*, (Journal of Chromatography Library, Vol. 39A), Elsevier, Amsterdam, 1988, Ch. 1 and 2, pp. 1–80, 81–143.
5 L. Yago, *Int. Lab.*, Nov./Dec. (1985) 40.
6 J. V. Pivnichny, A. A. Lawrence and J. D. Stong, *J. Chromatogr. Sci.*, 25 (1987) 181.
7 M. W. F. Nielen, A. J. Valk, R. W. Frei, U. A. Th. Brinkman, Ph. Mussche, R. de Nijs, B. Ooms and W. Smink, *J. Chromatogr.*, 393 (1987) 69.
8 J. B. Lecaillon, A. Sioufi, C. Souppart and J. P. Dubois, *Chromatographia*, 24 (1987) 876.
9 M. Broquaire and P. R. Guinebault, *J. Liq. Chromatogr.*, 4 (1981) 2039.
10 D. A. Schoeller, *Biomed. Mass Spectrom.*, 3 (1976) 265.