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ON-LINE LOW-LEVEL RADIOMETRIC DETECTION OF [^{14}C]REMOXIPRIDE IN LIQUID CHROMATOGRAPHIC EFFLUENTS

APPLICATION TO URINE SAMPLES

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SUMMARY

A method for on-line radiometric detection in liquid chromatography (LC) is described that permits the detection of low levels of radioactivity in LC effluents by using solvent segmentation and storage of the segmented effluent in a capillary storage loop.

The object of the method is to make the flow-rate in the on-line radioactivity monitor (and hence the mean residence time in the monitor) independent of the separation process. Therefore, after storage of the complete chromatogram, the segmented effluent is led through the monitor at flow-rates that can be chosen according to the residence time desired for accurate and precise radioactivity determination. In this system, it is possible to use a flow-cell volume small enough to preserve the chromatographic integrity, while maintaining the possibility of increasing the counting time.

Tests have been performed on the reproducibility of ^{14}C detection and the influence of the flow-rate through the monitor on the standard deviation in ^{14}C peak area and, thus, on the detection limit, using ^{14}C-labelled remoxipride. As an application, analyses of urine samples for [^{14}C]remoxipride and one of its potential metabolites are reported.

INTRODUCTION

The advantages of using radionuclide-labelled compounds in metabolism and trace recovery studies are well recognized. Detection is selective and sensitive, the sensitivity depending on the background level, counting efficiency and counting time of background and sample. For the determination of groups of structurally similar analytes, such as metabolites, degradation products and by-products, the coupling of liquid chromatographic (LC) techniques with radiometric detection has become

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a promising approach[1]. The radioactivity monitoring of LC effluents can be performed by off-line or on-line techniques.

In off-line counting, the LC effluent is fractionated. After mixing with a suitable liquid scintillator, the fractions are counted in a liquid scintillation counter. Obviously this method has the advantage of a free choice of counting time, depending on the radioactivity level and the standard deviation desired. The major drawback is the time-consuming procedure, which can easily lead to erroneous results and difficulties with automation and data acquisition. Also, the resolution depends on the fraction size chosen. Further, the reproducibility of the fraction collection becomes problematic with eluents that contain low percentages of organic solvents[2].

Two alternatives have been developed that permit the on-line radioactivity determination in LC effluents.

(1) Heterogeneous counting, in which the effluent is led through a radioactivity monitor with a flow cell packed with solid scintillator material. In this method, problems such as pressure build-up across the cell may arise, which will lead to leakage. Moreover, for some applications, irreversible adsorption of compounds on the scintillator material occurs. With radiolabelled compounds, this will lead to an undefined increase in the background, which hampers accurate and precise low-level radioactivity detection. Further, the counting efficiency for ^{3}H is low ($< 1\%$)[3].

(2) Homogeneous counting, in which the effluent is continuously mixed with a liquid scintillator before passing through the radioactivity monitor. In this instance no pressure build-up occurs but, as with heterogeneous counting, the counting time depends on the flow-rate (F) in the monitor and the flow-cell volume (V) as it equals the mean residence time t_d in the monitor:

$$t_d = V/F \tag{1}$$

In optimization of on-line radioactivity detection, one should focus on the increase in counts/peak in the radiogram recorded, as calculated from

$$\text{Counts} = EBt_d \tag{2}$$

where E is the counting efficiency and B the amount of radioactivity injected (Bq). For low beta-emitters, such as ^{14}C or ^{35}S, E values of over 0.5 are normally obtained. Therefore, as mentioned by Reeve and Crozier[4], little gain in sensitivity can be expected by increasing it. In general, a lower but constant E value is to be preferred over a high but varying performance.

The counting time, t_d, can be increased via the flow-cell volume, V. However, this will lead to a decrease in resolution. As a rule of thumb, the flow-cell volume should be less than one third of the volume standard deviation of the peak of interest[5]. Hence one should increase t_d while keeping V sufficiently low.

In a previous study we showed the possibility of increasing t_d independent of the separation process (that is, independent of the flow-rate, F, used for the separation process and the addition of liquid scintillator) by using solvent segmentation of the LC effluent[6]. Segmentation helps to suppress band broadening in the relatively large flow-cell volume and connecting tubing of the on-line radioactivity detector, as first demonstrated by Bakay[7], who succesfully used a semi-solid acrylamide gel as the segmentor. In our approach, two radiograms can be recorded, direct or reverse.

In the direct mode, after UV detection the water-containing effluent is mixed with a water-immiscible liquid scintillator. This scintillator has three functions: as an extraction medium for low- to medium-polarity compounds from the effluent into the scintillator; as a detection medium for the extracted radiolabelled compounds; and to segment the LC effluent with water-immiscible organic solvent plugs, which suppresses band broadening of the effluent in the capillaries. As a result, a solvent-segmented flow pattern is obtained, which is led through the radioactivity monitor and the direct radiogram is recorded at the combined flow-rate of eluent and scintillator (typically 2.0 ml/min). After detection, this mixture is stored in a capillary storage loop.

In the reverse mode, after storage of the complete chromatogram it can be reintroduced into the radioactivity monitor at a flow-rate independent of the chromatographic conditions, and so, as with off-line counting, in the reverse radiogram the counting time, t_d, can be chosen to match the radioactivity level (as recorded in the direct chromatogram) and the desired sensitivity. Owing to the increase in t_d, an increase in peak area and, hence, an increase in sensitivity are obtained relative to the direct radiogram. Further, it would be possible to program the flow-rate for the reverse measurement according to the radioactivity distribution, as recorded in the direct run, and/or by programming regions of interest in the chromatogram. This will be dealt with in a subsequent paper. In our opinion, this approach is more versatile than the alternative of lowering the flow-rates of the eluent and scintillator during the separation. In the latter instance much time will be wasted for regions where no radioactivity elutess. Also, the possible gain in t_d will be limited, owing to band broadening in the column, connecting tubes and detectors at very low flow-rates.

To test this system, the determination of polar [^{14}C]remoxipride, a recently developed antipsychotic agent[8–10], in human urine samples from volunteers who had been given a dose of [^{14}C]remoxipride was performed. A pharmaceutical compound was chosen because most applications of radio-LC refer to pharmaceutical and biomedical research[1]. Further, we wanted to demonstrate the applicability of this technique to polar compounds, *i.e.*, compounds that are poorly extractable into the scintillator phase under the separation conditions used.

EXPERIMENTAL

Apparatus

A schematic diagram of the system is given in Fig. 1. The set-up consists of a Model 3B dual-head pump (Perkin-Elmer, Norwalk, CT, U.S.A.), a Model 7126 injector (Rheodyne, Cotati, CA, U.S.A.), used with a Rheodyne 7163 solenoid valve kit, a Uvikon 725 UV detector (Kontron, Zurich, Switzerland) and a Minipuls Model HP4 peristaltic pump (Gilson Medical Electronics, Villiers-le-Bel, France), used for adding aqueous sodium hydroxide to the effluent. The combined flows are mixed with the scintillator through a T-piece. The segmented flow then passes a Model 12611 eight-port switching valve (Valco, Houston, TX, U.S.A.) with an air activator and a Rheodyne 7163 valve kit. Following the extraction coil, the flow is led through the radioactivity monitor, which consists of a laboratory-built light-tight housing, an Isoflo flow cell (Nuclear Enterprises, Edingburgh, U.K.) with a geometric volume of

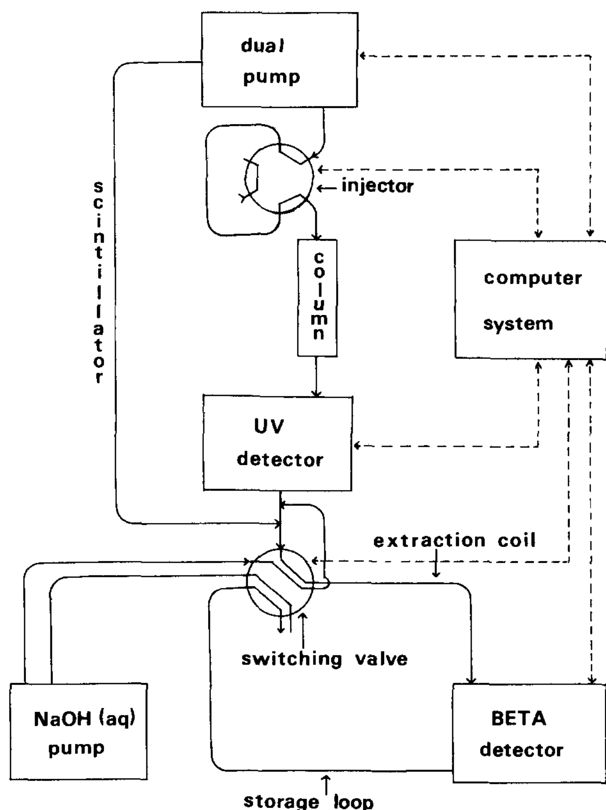


Fig. 1. Schematic diagram of LC equipment with on-line radioactivity monitor and extraction/storage system for the effluent.

63 μl, two Model 8575 photomultiplier (RCA, Harrison, NJ, U.S.A.), a Model 456 high-voltage power supply (Ortec, Oak Ridge, TN, U.S.A.), two PM bases (Ortec 265), two magnetic shieldings (Ortec 218), two scintillator pre-amplifiers (Ortec 113), two Model 2110 timing filter amplifiers (Canberra, Meriden, CT, U.S.A.), and two constant-fraction discriminators (Canberra 1428A). The segmented eluate is stored in a stainless-steel tube (50 m × 1/16 in. O.D. × 1.0 mm I.D.). The chromatographic apparatus is controlled with laboratory-devised software by a computer system consisting of a DATA-RAM LSI-11/2 computer with a DEC LSI-11/2 microprocessor under RT-11 (Digital Equipment, Maynard, MA, U.S.A.). A Model 862 interface (Nelson Analytical, Cupertino, CA, U.S.A.), a Model 910 terminal (Televideo, Sunnyvale, CA, U.S.A.), a Model MX-100 III printer (Epson, Nagano, Japan) and a laboratory-built coincidence counter/time interface are also used.

Modifications introduced during the investigation refer to alteration of the flow-rate range of the Perkin-Elmer dual-head LC pump and the extraction procedure of the LC effluent with the water-immiscible liquid scintillator:

(a) The lowest possible flow-rate of the Perkin-Elmer LC pump was changed electronically from 100 to 5 μl/min, thereby increasing the maximum residence time in the flow cell of the radioactivity monitor from 0.72 to 14.4 min.

(b) In order to increase the extractability of the compounds of interest into the scintillator phase, it was found necessary to increase the pH of the effluent to >9 before adding the scintillator. This was accomplished by post-column addition of an aqueous 1 *M* sodium hydroxide solution with a peristaltic pump. In the present set-up it is not possible to control the flow-rate of this pump externally by the computer system. However, by using the eight-port Valco switching valve it was possible to incorporate this pump in a recycling system. During the separation process (direct measurement), the sodium hydroxide solution is added to the effluent; during the reverse measurement, the solution automatically flows back into the aqueous sodium hydroxide reservoir.

(c) In contrast to previous work[6], the 2-μm in-line solvent filter, intended to increase the yield of analytes extracted into the scintillator, could not be used, because the combination of scintillator and sodium hydroxide solution led to irreversible salt formation on the filter and, thus, to high back-pressure and leakages. Fortunately, omitting the 2-μm filter did not adversely influence the extraction yield of the compound of interest.

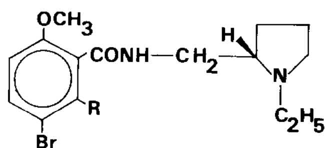
Chemicals and standards

The chromatographic conditions were determined with Brownlee Cyano Spheri-5 cartridge columns (Brownlee Labs., Santa Clara, CA, U.S.A.), doubly distilled water, acetonitrile (HPLC grade) (Fisons, Loughborough, U.K.), acetic acid (Baker Analyzed reagent) (Baker, Deventer, The Netherlands) and tetra-*n*-butylammonium bromide (TBA) (HPLC grade) (Fisons). Ready Solv NA (Beckman, Fullerton, CA, U.S.A.) was used as a water-immiscible liquid scintillator.

For absolute activity determinations on standards, urine samples and injected samples, Instagel (United Technologies Packard, Warrenville, IL, U.S.A.) was used as a water-miscible scintillator.

Remoxipride, FLA 797 (a potential metabolite of remoxipride[11]), [^{14}C]remoxipride (specific activity 1060 MBq/mmol, radiochemical purity 96.7%) and urine samples were gifts from ASTRA Alab (Södertälje, Sweden). Structural formulae of remoxipride and FLA 797 are given in Fig. 2.

All standards and stock solutions were kept at 4°C. Urine samples (taken 4–6 h after administration) were stored at -18°C. Two methods of sample treatment were used: enzymatic β-deglucuronidation was carried out by incubation of 1.0 ml of urine in 1.0 ml of 0.02 *M* acetate buffer (pH 4.5) containing 10 mg of Lipton



	R:
remoxipride	$-OCH_3$
FLA 797	$-OH$

Fig. 2. Structural formulae of remoxipride [R = OCH_3; (−)-form, as shown] and FLA 797 (R = OH; racemic mixture).

acetone powder Type I (Sigma, St. Louis, MO, U.S.A.) for 24 h at 37°C; acid hydrolysis was performed by heating 1.0 ml of urine in 1.0 ml of 12 *M* hydrochloric acid (Merck, Darmstadt, F.R.G.) at 100°C for 1 h. Both hydrolysates were extracted with chloroform (Baker Analyzed reagent) after adjustment of the pH with aqueous sodium hydroxide (Baker grade) to pH 11. The organic phase was separated and evaporized under a gentle stream of nitrogen. The residue was diluted in 200 μl of eluent and volumes of 23 μl were injected.

RESULTS AND DISCUSSION

LC of remoxipride and FLA 797

FLA 908, a phenolic isomer of FLA 797, has earlier been identified as a human remoxipride metabolite and the compound has also been determined in urine after enzymatic hydrolysis[12]. For this determination a liquid chromatographic system giving peak compression effects[13] was applied together with UV detection. In our procedure, ion-pair chromatography on a cyano-bonded column is used, which performs better than an RP-18 column. On the cyano-bonded column, the retention time of remoxipride was decreased from 21 to 3.6 min by the addition of 1.0 *M* TBA to the eluent. This may be due to a competition between the analyte and TBA for the residual silanol groups on the packing material. This hypothesis is supported by the fact that the more polar FLA 797 had a longer retention time than remoxipride.

Fig. 3 shows a chromatogram of remoxipride and FLA 797 with UV detection. Both peaks show tailing, as amine-containing (basic) pharmaceuticals often do.

Post-column addition of aqueous sodium hydroxide

Because of the small range of the beta particles emitted in the radioactive decay of ^{14}C (ref. 14), it is desirable that the ^{14}C-labelled compounds be in the scintillator phase in order to obtain an adequate counting efficiency. Therefore, in batch experiments, the influence of the pH of the eluent on the extraction of remoxipride and FLA 797 was tested, using *o*-xylene or dichloromethane as the extraction medium and UV detection.

At pH < 6, the extraction yield was $<0.1\%$ for both analytes. At pH 11.5, extraction was almost quantitative for both analytes, both in the presence and ab-

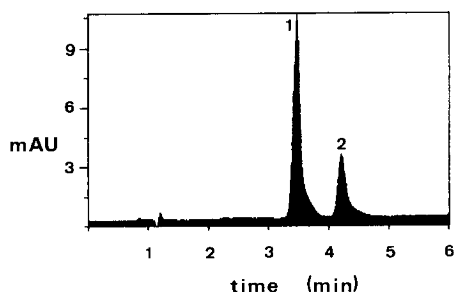


Fig. 3. Chromatogram of (1) remoxipride and (2) FLA 797 with UV detection. Sample, $6.87 \cdot 10^{-5}$ *M* (1.58 nmol) remoxipride and $2.15 \cdot 10^{-5}$ *M* (0.49 nmol) FLA 797 in 23 μl; column, Brownlee Cyano Spheri-5 (100 × 4.6 mm I.D.); eluent, acetonitrile–water–acetic acid (40:60:1, v/v/v) (pH 3.1) containing 1.0 m*M* TBA; flow-rate, 1.0 ml/min; detection, 233 nm.

sence of TBA. For remoxipride the pH effect is demonstrated in Fig. 4B and C. Fig. 4A shows the UV chromatogram for a 1.55 nmol (1640 Bq) injection of [^{14}C]remoxipride. Fig. 4B and C show the corresponding radiograms (direct measurement) without and with post-column addition of sodium hydroxide, respectively. Both radiograms were smoothed by fast Fourier transform (FFT) filtering. The counting efficiencies (as calculated from eqn. 2) are 0.04 and 0.65, respectively.

Surprisingly, an additional advantage of the post-column increase in the pH is the more rapid formation of a clear segmentation pattern for the scintillator and effluent.

Linearity and reproducibility of UV and ^{14}C detection

The linearity of the system for direct measurements was tested by varying the amounts of [^{14}C]remoxipride and FLA 797 injected. For UV detection, the plots

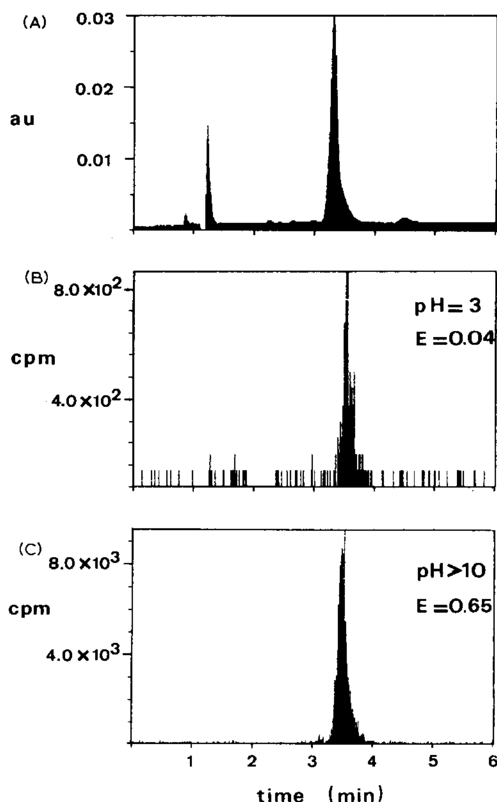


Fig. 4. UV chromatogram and direct radiograms for 1640 Bq (1.55 nmol) [^{14}C]remoxipride injections without and with addition of aqueous sodium hydroxide to the eluate. For convenience, y values from the radiograms are normalized to cpm, according to cpm = $(60/ST)$ × counts (ST = sampling time in seconds, counts = counts per sample). The radiograms are smoothed by FFT filtering. Liquid scintillator, Ready Solv NA (flow-rate = 1.0 ml/min). For chromatographic conditions, see Fig. 3. (A) UV chromatogram; (B) direct radiogram without addition of sodium hydroxide; ^{14}C peak area equals 121 ± 12 counts; (C) direct radiogram with addition of sodium hydroxide (0.23 ml/min); ^{14}C peak area equals 1671 ± 41 counts.

were linear over 2.5 orders of magnitude for remoxipride and FLA 797 (r = 0.9999, n = 8). At concentrations above $0.3 \cdot 10^{-3}$ M fronting of the peaks occurred. The reason for this is unknown. It could be attributed to overloading of the column and/or the presence of an insufficient excess of the ion-pair reagent relative to the analyte.

For radioactivity detection, the plot was linear over 1.5 orders of magnitude (r = 0.9995, n = 20). The upper limit was determined by the maximum concentration that could be prepared from the stock solution. It should be borne in mind that for radioactivity measurements, weighted regression analysis has to be performed because the statistical weights of the data vary appreciably[15].

To test the reproducibility, a sample of [^{14}C]remoxipride (4600 Bq, 4.34 nmol) was injected repeatedly (n = 9) with direct and reverse measuring times of 6.0 and 60.0 min, respectively. For UV detection, the mean peak area was 118.6 mAU · ml [relative standard deviation (R.S.D.) = 1.62%]. For ^{14}C detection, the mean peak areas were 4846 (R.S.D. = 2.07%) and 61 030 (R.S.D. = 1.77%) counts for direct and reverse measurements, respectively. From these figures it can be concluded that the reproducibility of ^{14}C detection almost equals that of UV detection.

It should be noted that, owing to the statistical nature of the radioactive decay, with known values for mean peak areas and number of measurements, the R.S.D. can be calculated for these areas[15]; values of 1.36% and 0.39% were found for the direct and reverse measurements, respectively. It is of interest to compare these expected R.S.D. values (*i.e.*, values calculated if the deviations were determined by the radioactive decay alone) with the experimental values. As expected for these high-activity injections, the experimental R.S.D. is determined by factors others than the radioactive decay, such as fluctuations in amount injected, counting efficiency or flow-rate through the radioactivity monitor.

With regard to the peak areas, the peak areas from the reverse measurements are about 12.6 times larger than those from the direct measurements, although t(rev.) is exactly ten times t(dir.). This can be attributed to differences in the flow-rates through the radioactivity monitor (0.195 and 2.17 ml/min, respectively) and differences in the effective cell volume of the monitor. The latter does not necessarily equal the geometric volume but is a function of the flow-rate (and hence the flow pattern) through the cell[16]. In this instance the values 62 and 72 μl apply to direct and reverse measurements, respectively.

Finally, from the ratios of peak areas for direct and reverse measurements it can be calculated that the ^{14}C counting efficiency is not adversely influenced by the storage process of the segmented effluent.

Influence of the reverse flow-rate on ^{14}C peak area

For the high-activity injections of [^{14}C]remoxipride it was concluded that the R.S.D. values of ^{14}C peak areas were determined mainly by the experimental conditions (*i.e.*, reproducibility of injection, flow-rate through the radioactivity monitor and counting efficiency). For low-activity injections, the R.S.D. of ^{14}C peak areas are expected to be determined largely by the statistical nature of the radioactive decay[17]. This is especially true for the direct measurements, where small peak areas are obtained, owing to the relatively short counting time.

For the determination of the relationship between ^{14}C peak areas, or their

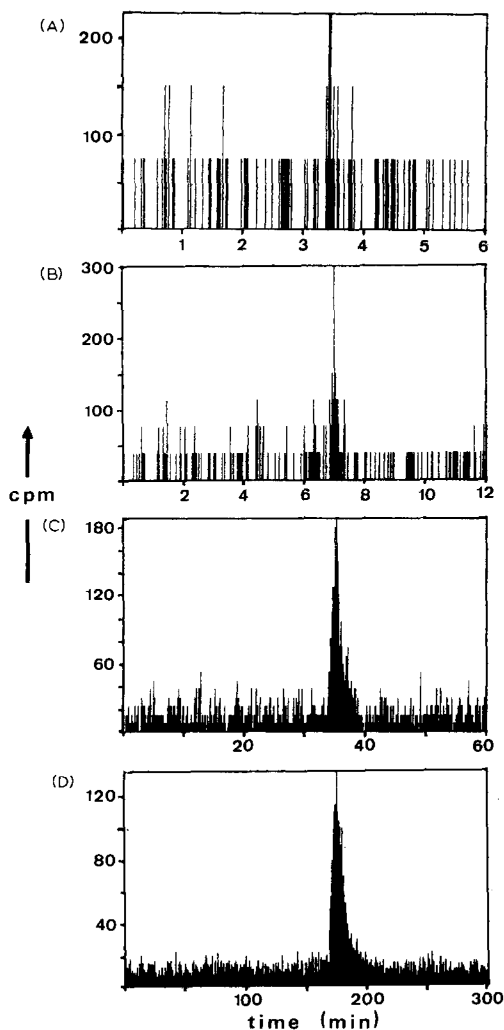


Fig. 5. Radiograms for 16 Bq (15 pmol) injections of [^{14}C]remoxipride as a function of the flow-rate (counting time) in the radioactivity monitor. For comparison, raw data (data not FFT filtered) are given. The y values are normalized to cpm. For chromatographic conditions, see Fig. 4C. (A) direct radiogram, F(dir.) = 2.05 ml/min (t_d = 0.03 min); (B) reverse radiogram, F(rev.) = 1.00 ml/min (t_d = 0.06 min); (C) radiogram, F(rev.) = 0.20 ml/min (t_d = 0.37 min); (D) radiogram, F(rev.) = 37 μl/min (t_d = 2.02 min).

R.S.D., and the flow-rate through the radioactivity monitor (and, thus, the counting time), repetitive injections of 16 Bq (15 pmol) of [^{14}C]remoxipride were made while varying the reverse flow-rate from 5 to 2060 μl/min.

As examples, Fig. 5B–D show some reverse radiograms obtained at different flow-rates. Fig. 5A gives the direct radiogram. For comparison, raw data, *i.e.*, data not FFT filtered, are given. The increase in ^{14}C peak area, *i.e.*, the decrease in the detection limit, is clearly seen.

A more quantitative way of demonstrating the influence of the reverse flow-

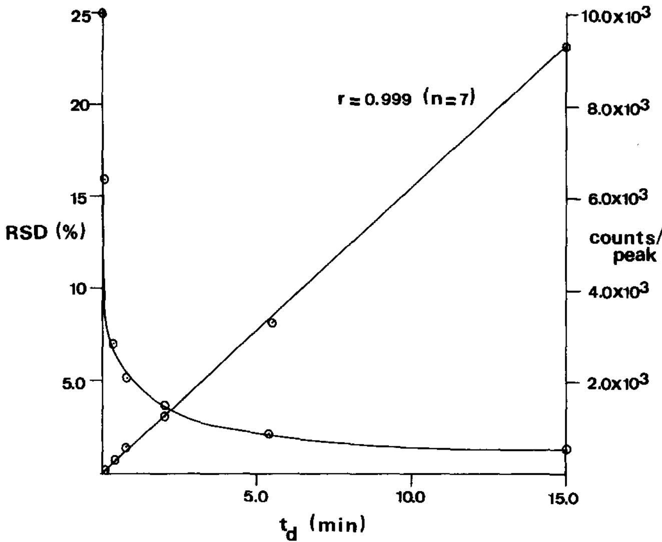


Fig. 6. Relative standard deviation of peak area as a function of the counting time and peak area as a function of the counting time for a 16 Bq (15 pmol) injections of [^{14}C]remoxipride. Data were taken from Fig. 5A–D and similar radiograms.

rate on the detection limit is to express the R.S.D. of the ^{14}C peak area as a function of the mean residence time, t_d, in the radioactivity monitor. This is illustrated in Fig. 6. Data were taken from the radiograms given in Fig. 5 and similar radiograms. The R.S.D. values were calculated according to:

$$\text{S.D.} = c_p + \left[\frac{(t_w)}{(t_b)} \cdot (c_b)^{1/2}\right]^{2\ 1/2} \tag{3}$$

$$\text{R.S.D.} = (\text{S.D.}/c_n) \cdot 100\% \tag{4}$$

where c_p = peak area (gross counts); c_n = peak area (net counts); c_b = background (counts recorded in time t_b); and t_w = peak width (s).

From Fig. 6, it is clear that there is a 5-fold increase in sensitivity on comparing the direct measurement (t_d = 0.03 min) with the reverse measurement (t_d = 0.73 min). Increasing t_d to 2.5 min yields only a small further gain in sensitivity (2-fold).

As is usual in radioactivity detection, the R.S.D. varies inversely with $(t_d)^{0.5}$, as can be verified from the data given in Fig. 6. Also, it is of interest to test the linearity of the peak area as a function of t_d. A correlation coefficient r = 0.9994 (n = 8) is calculated with weighted linear regression analysis. Deviations from the fitted line (see Fig. 6) can be ascribed to pumping inaccuracies at low flow-rates and the influence of flow-rate on the effective flow-cell volume mentioned above. Detailed experiments have shown that, for flow-rates below 15 μl/min, the pumping accuracy becomes problematic, although the reproducibility is satisfactory[18]. In other words, at low flow-rates the mean residence time, t_d, is still reproducible but is inaccurate.

Analysis of urine samples

As an application, some experiments were performed with human urine samples. The enzymatic or acid hydrolysis of the samples (see *Chemicals and standards*) did not adversely affect the percentage extraction of ^{14}C activity into the cloroform layer. From this, one may conclude that remoxipride and its metabolites are present in the samples in the free form. Consequently, samples of pH > 10 were extracted directly with chloroform.

An example is given in Fig. 7. The total activity injected was 20 Bq. The UV chromatogram is shown in Fig. 7A. The direct (t_d = 0.03 min) and reverse (t_d = 4.93 min) radiograms are shown in Fig. 7B and C, respectively. Fig. 7D shows the

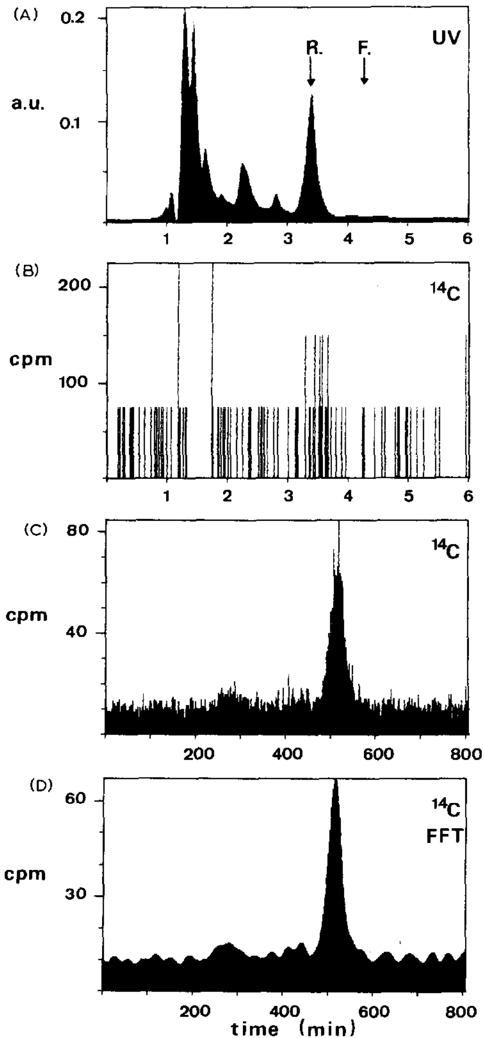


Fig. 7. (A) UV chromatogram and (B) direct and (C) reverse radiograms of a urine sample. (D) Reverse radiogram after smoothing the data by FFT filtering. The y values of the radiograms are normalized to cpm. For chromatographic conditions, see Fig. 4C.

reverse radiogram after smoothing the data by FFT filtering. The effect of flow-rate through the radioactivity detector is clearly seen. At t_d = 1.5–2.0 min some ^{14}C activity is present, which corresponds to 15 ± 5% of the [^{14}C]remoxipride peak area. From the ^{14}C detection limit it can be calculated that, in all samples, the amount of FLA 797 (if present) is less than 5% of the remoxipride dose given.

In most instances the peak shape for remoxipride is poor owing to overloading of the analytical column and/or the presence of an insufficient excess of the ion-pair reagent relative to the amount of remoxipride. This was caused by the relatively low specific activity of the [^{14}C]remoxipride dose given (prior to administration, [^{14}C]remoxipride of high specific activity was diluted about 650-fold with unlabelled remoxipride). As a result, the concentrations of remoxipride in the injected samples varied between 0.1 and 0.5 m*M*, with a low activity (10–50 Bq). In principle, this problem can be avoided by reducing the total dose while keeping the ^{14}C dose at the same level. In this way, a solution with a higher amount of ^{14}C activity can be prepared for injection, thereby minimizing the analysis time required for accurate and precise radioactivity determination.

CONCLUSIONS

A versatile and precise method has been developed for an on-line increase in the counting time in radio-LC. Segmentation of the LC effluent with a water-immiscible liquid scintillator permits the storage of the effluent with negligible peak broadening. Linear relationships between ^{14}C peak area and the mean residence time, t_d, and between the reciprocal of the R.S.D. of peak area and t_d were obtained.

Problems encountered during this study were (a) reduced accuracy of the pump at flow-rates of less than 15 μl/min and (b) owing to the very low specific activity of the [^{14}C]remoxipride dose administered to the volunteers it was not possible to prepare a more concentrated solution from the urine samples for injection. Therefore, long reverse measurements were necessary for accurate and precise radioactivity detection. This can be avoided by using as high as possible specific activities (not diluted with non-labelled remoxipride) for administration.

Experiments on another potential drug indicate that the present method can be used generally in metabolism studies on basic pharmaceuticals containing a tertiary amino group. The extractability of these compounds into the water-immiscible scintillator increases with increasing pH of the eluent.

For convenience, the post-column increase in the pH of the eluent can be accomplished by in-line generation of hydroxy anions on an anion-exchange column and eluent splitting, as described by Jansen *et al.*[19]. The obvious advantage is that the peristaltic pump can be omitted. In the future, this aspect will be explored, and also the possibility of post-column ion-pair extraction of basic pharmaceuticals with a suitable ion-pair reagent[20]. This reagent can be added to the eluate with a peristaltic pump, or it can be present in the eluent. In the latter instance, no extra pump would be needed.

A further improvement is the use of so-called "regions of interest". During the reverse measurement, only these regions in the stored effluent are transported through the radioactivity monitor at low flow-rates, the remainder being pumped through the monitor at a normal flow-rate. Hence the total analysis time is considerably shortened while long counting times in the regions of interest are maintained.

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