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Appropriate column configurations for the rapid analysis and semipreparative purification of the radiolabeled drug flutamide by high-performance liquid chromatography

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Abstract

Flutamide, marketed as Eulexin, is used for treatment of metastic prostatic carcinoma. Purity of a radiolabeled batch for metabolism studies was first determined by reversed-phase HPLC on a 5 μm, 150×4.6 mm analytical column. The separation was then scaled up to give a semipreparative column (5 μm, 250×10 mm) purification procedure. Fraction analysis was done on a short rapid analysis (5 μm, 50×3.0 mm) column. Analysis of the final product was performed on the analytical column. All columns were YMC-Pack ODS-AQ. The analytical work involved large mass injections in order to have the required amounts of radioactivity needed for accurate impurity profile determinations, and the preparative work involved masses much larger than the calculated scale-up values. Ultraviolet and radiochromatograms of the drug on the various column configurations are compared. A 95.7% recovery of product was obtained, with radiochemical purity increased from 95.0 to 99.8%.

Keywords: Preparative chromatography; Pharmaceutical analysis; Flutamide

1. Introduction

Radiolabeled compounds are essential tools in modern drug research and development and are particularly valuable in drug metabolism studies [1]. Pharmaceutical radiochemistry groups are routinely required to provide milligram-to-gram amounts of structurally diverse compounds. These compounds must be of high radiochemical and chemical purity in order to generate meaningful data. These compounds span the range from ''development'' compounds which are very well characterized to ''discovery'' compounds for which analytical reference standards are not yet available. High purity is most effectively achieved using liquid chromatography.

The analytical and purification methods must be developed and carried out in extremely short timespans. The methods are not meant for routine use beyond our radiochemistry laboratory, and thus extensive method validation and isolation/identification of impurities is not required. Any particular radiolabeled drug may in fact be synthesized and purified only once.

Dolan has recently summarized the important aspects of changing column conditions in methods development [2]. We incorporate those involving use of (i) intermediate columns for initial method development, (ii) changing column length and/or diameter to shorten run time and (iii) increasing flow-rate to trade excess resolution to decrease runtime.

Our usual procedure makes use of standard high-performance liquid chromatography (HPLC), semi-

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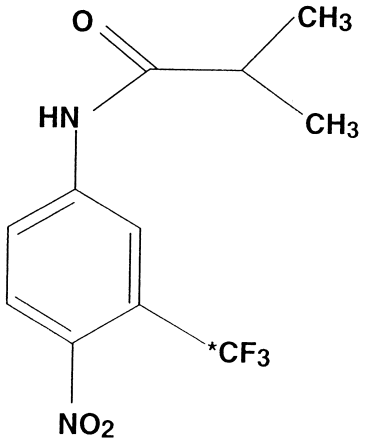

Fig. 1. Structure of radiolabeled [^{14}C]flutamide.

preparative HPLC, and rapid analysis HPLC. We use columns of three different configurations, each appropriate to the particular chromatographic need at that stage of the process. We also routinely inject solute masses much larger than usually associated with these columns.

We here describe a representative purification procedure. Our goal was the rapid purification of a crude batch of flutamide, marketed as Eulexin for treatment of metastic prostatic carcinoma [3]. The structure is shown in Fig. 1.

2. Experimental

2.1. Reagents and chemicals

All solvents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Acetonitrile was HPLC grade, triethylamine was reagent grade, and glacial acetic acid was A.C.S. Plus grade.

The liquid scintillation cocktail used in the radioactivity detector was In-Flow 3 (IN/US Systems, Tampa, FL, USA) at a flow-rate twice that of the HPLC mobile phase.

The buffer phase, 0.05 *M* triethylamine acetate (TEAA), pH 3.5, was prepared by adding 7.0 ml triethylamine to 800 ml distilled water, adjusting pH by dropwise addition of glacial acetic acid, and diluting to volume with distilled water. Organic and buffer phases were added to different reservoirs and were mixed by the HPLC pump.

2.2. Equipment

2.2.1. Analytical HPLC

The analytical HPLC system (Waters, Milford, MA, USA) consisted of a Model 600 multisolvent delivery system, a Model 717 Plus autosampler, and a Model 2487 dual wavelength absorbance detector set at 254 nm. The data processing system was a Millennium 32 Chromatography Manager V3.05.01 with System Suitability Option. Data acquisition was at one point per second. Column temperature was controlled at 26±1°C using a Croco-Cil column heater (Astec, Whippany, NJ, USA).

The eluate from the UV detector is mixed with liquid scintillation cocktail. The homogeneous mixture passes through a flow cell where radioactivity is continuously monitored and recorded. The radiochemical detection system (IN/US Systems) was a Beta-Ram Model 2B with software Win-Flow V1.2. The flow cell volume was 500 μl and the time constant was 6 s.

2.2.2. Preparative HPLC

The preparative HPLC system (Waters) consisted of a Delta Prep 4000 delivery system, a Model 486 tunable absorbance detector set at 254 nm, a 746 data module, and a Model 7125 injector (Rheodyne, Cotati, CA, USA), with a 5.0-ml loop.

2.3. Columns

All columns were YMC-Pack ODS-AQ (YMC, Wilmington, NC, USA). The ''analytical'' column was 150 mm×4.6 mm, the ''semipreparative''column was 250 mm×10 mm, and the ''rapid analysis'' column was 50 mm×3.0 mm. All contain C_{18} ligands bonded to 5 μm 120 Å silica, followed by a hydrophilic endcapping [4].

3. Results and discussion

3.1. Initial analytical HPLC

Fig. 2 shows optimized analytical UV chromato-

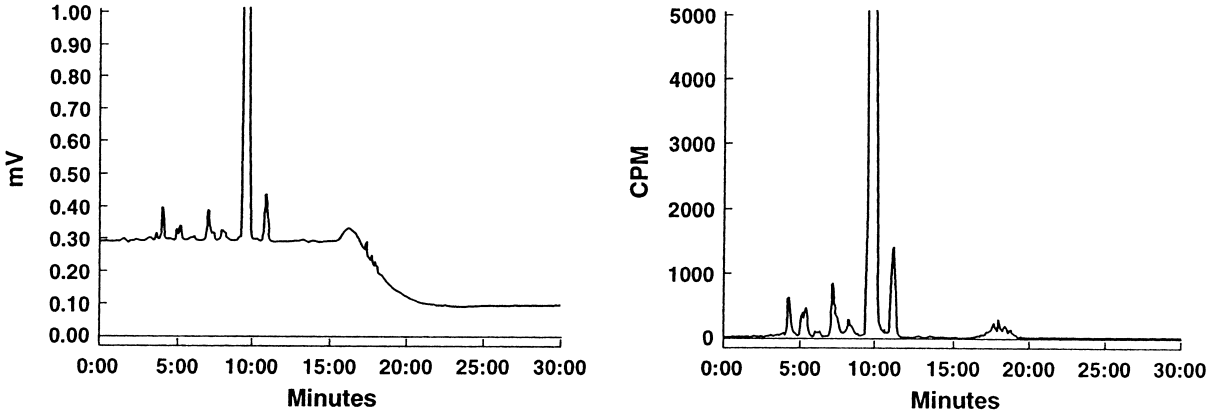

Fig. 2. Analytical UV chromatograms (left) and radiochromatograms (right) of crude [^{14}C]flutamide. Column dimensions are 150×4.6 mm.

grams and radiochromatograms of the crude batch on the standard column. Chromatographic conditions are 0.05 *M* TEAA, pH 3.5–CH_3CN (50:50) for 10 min followed by a step gradient to CH_3CN. Flow-rate was 1.0 ml/min. The step gradient is necessary in the analysis of radiolabeled compounds to remove any highly retained impurities and thus assure that all injected radioactivity is eluted from the column.

From the radiochromatogram, the main peak contains 95% of the total radioactivity.

From the UV chromatogram, the main peak contains 97% of the total integration.

The resolution of the main peak from its closest impurities is calculated from the equation [5]:

$$R_s = 2\Delta t_R/(w_1 + w_2)$$

For the UV chromatogram, the resolution of the main peak from the earlier-eluting impurity is 2.2 and from the later-eluting impurity is 1.8. The corresponding values from the radiochromatogram are 1.6 and 1.6. Some degradation of resolution is to be expected on mixing the UV-cell eluent with liquid scintillation cocktail and passing it through the 500 μl radioactivity detector flow cell. Preparative fractions are collected based on the UV chromatogram, and thus the correspondence of the UV and radioanalysis (RA) peaks for this batch would appear to lead to a good purification.

Most reversed-phase columns will handle about 1–10 μg of sample per gram of packing material [6]. The injected volume was chosen to provide a very substantial amount of radioactivity (≈1 μCi) so as to maximize the determination of minor impurities. The analytical injection (1.7 μCi) in 5 μl CH_3CN is 46 μg, about twice the maximum value.

The Millennium System Suitability software uses the following equations to measure the quoted parameters:

Capacity factor: $k' = t_R/V_0 - 1.0$ where t_R = retention time and V_0 = void volume time.

Asymmetry: $A_s = A/B$ where A = time from peak retention time to width end point at 4.4% of peak height and B = time from peak retention time to width start point at 4.4% of peak height.

USP tail: $T = W/2F$ where W = peak width at 5% of peak height and F = time from width start point to retention time at 5% of peak height.

Plate count: $N = 16(t_R/W)^2$ where t_R = retention time and W = peak width at baseline determined by tangents drawn to 61% of peak height.

For the UV main peak the calculated parameters were $k' = 5.3$, $A_s = 1.3$, USP tail = 1.2, and $N = 1150$.

3.2. Preparative HPLC

From the usual column scale-up equations [7] the preparative (prep.) flow-rate and mass load can be calculated:

Prep. flow-rate = analytical flow-rate

· (prep. diameter/analytical diameter)^2

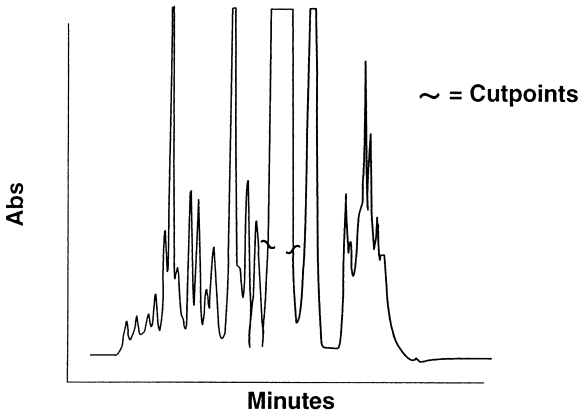

Fig. 3. Preparative UV chromatogram of a 37.7 mg injection of crude [^{14}C]flutamide. Column dimensions are 250×10.0 mm.

Prep. mass load = analytical mass load

· (prep. I.D./analytical I.D.)^2

· (prep. length/analytical length)

The calculated values are 4.7 ml/min and 362 μg, respectively. As the total batch size was 377 mg, the injected mass would have to be much larger than calculated in order to purify the batch in a reasonable number of injections. Therefore a hundredfold increase in mass size was evaluated on the semi-preparative column.

The bulk batch (13.9 mCi, 377 mg) was dissolved in 2.0 ml CH_3CN. Fig. 3 shows the UV chromatogram of an initial 200 μl (1390 μCi, 37.7 mg) injection. The main peak was hand-collected using the cut points shown. The flow-rate was 5.0 ml/min. The step gradient to CH_3CN was done at 20 min and reequilibration to starting solvent was done at 29 min. Note the good correspondence of the preparative and analytical chromatograms (Figs. 2 and 3). The preparative k' is 5.7, in good agreement with the standard analytical column 5.3 value.

The remaining batch was then purified in 200 μl increments. Each injection required 35 min, including column reequilibration. Thus all injections were complete in under 6 h.

3.3. Rapid analysis HPLC

Concurrently with the purification injections, the main fractions were assayed by ''rapid analysis'' HPLC on the 50 mm×3.0 mm column. Fig. 4 shows representative UV chromatograms and radiochromatograms. Flow-rate was 1.0 ml/min. The step gradient to CH_3CN was done at 3.0 min. Thus the time between injections was 17 min, including reequilibration. Thus all analytical injections could be completed in about 3 h. A 5 μl injection of the fractions was chosen to again give the radioactivity needed for good impurity determination. For the injection shown this corresponded to a 23 μg load, about six times the maximum. Measured peak parameters were A_s=1.18, USP tail=1.10 and N=

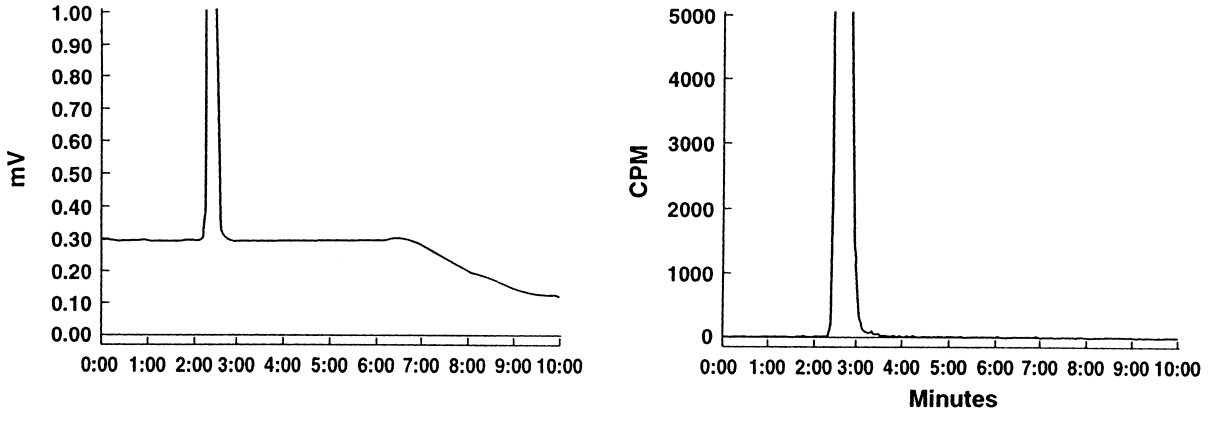

Fig. 4. Rapid analysis UV chromatograms (left) and radiochromatograms (right) of a representative isolated main fraction from a preparative purification injection. Column dimensions are 50×3.0 mm.

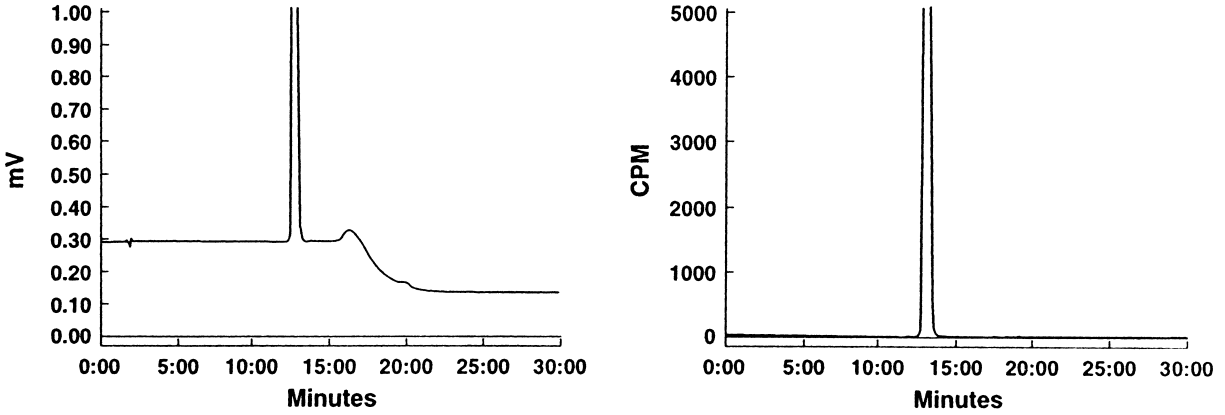

Fig. 5. Analytical UV chromatograms (left) and radiochromatograms (right) of final pure batch of [^{14}C]flutamide. Column dimensions are 150×4.6 mm.

2740. For this representative fraction chemical purity was 99.9% and radiochemical purity was 99.8%.

3.4. Final analytical HPLC

All main fractions were pooled, stripped of solvent by rotary evaporation, and dissolved in absolute ethanol to give the final batch. Storage of radiolabeled compounds in an organic solvent at the lowest possible temperature is common procedure to retard radiochemical decomposition. Because of the rapid purification procedure described, the fractions were stored (with refrigeration) in the more problematic reversed-phase mobile phase solvent system only for one night.

The final batch size was 13.3 mCi, representing a 95.7% recovery. It was analyzed on the analytical column used for the initial development work. Fig. 5 shows representative UV chromatograms and radiochromatograms. Chemical purity is 100%, and radiochemical purity is 99.8%. Injection size is 5 μl of ethanol bulk solution corresponding to 0.3 μCi and a still high but somewhat more realistic 8 μg mass load. The measured peak parameters are $A_s = 1.13$, USP tail = 1.08, and $N = 11\,800$.

4. Conclusion

A crude batch of radiolabeled flutamide was analyzed on a 150×4.6 mm column. It was then purified on a 250×10 mm column, with fraction analysis on a 50×3.0 mm column. Final batch analysis was done on a 150×4.6 mm column. Analytical injected masses were much larger than usual for these columns, with acceptable peak parameters being obtained. Preparative injections were completed in 6 h and fraction analysis in 3 h.

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