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IDENTIFICATION OF ERGOT ALKALOIDS WITH A PHOTOCHEMICAL REACTION DETECTOR IN LIQUID CHROMATOGRAPHY

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SUMMARY

An on-line photochemical reaction detector is described for the identification of ergot alkaloids by high-performance liquid chromatography. The fluorescence signal of alkaloids decreases within about 20 sec of irradiation and disappears selectively from complex chromatograms. The application of this principle to urine samples is described.

INTRODUCTION

Ergot alkaloids are an important group of pharmaceutically active substances. The identification of ergot alkaloids in a high-performance liquid chromatography (HPLC) chromatogram is usually difficult, but especially useful and necessary in complex matrices, such as biological materials. Blake *et al.*[1] noticed this difficulty in the quantitative analysis of lysergic acid dimethylamide (LSD). For HPLC, UV and fluorescence spectroscopy can be used for on-line identification. To enhance the reliability of this approach, an additional specific identification method is therefore desirable.

Recently, Twitchett *et al.*[2] have shown the feasibility of a photochemical reactor as an on-line detection system in liquid chromatography (LC). They mentioned very briefly the decrease in fluorescence of LSD on irradiation and the use of this phenomenon in the identification of LSD. In the present study we have investigated the application of a photochemical reaction detector in the qualitative determination of a wide range of ergot alkaloids.

Stoll and Schlientz[3] have investigated the photochemistry of ergot alkaloids and proposed the reaction shown in Fig. 1, whereby no fluorescence is observed for the lumi derivatives of ergot alkaloids. This loss of the fluorescence on irradiation with UV light has been investigated for ergot and 9,10-dihydroergot alkaloids and seems to be specific to this group of compounds. It can therefore be used for identification purposes. From these investigations it appears that the scheme in Fig. 1

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does probably not apply to the reaction occurring in the photochemical reactor. This belief is based on the observation that the dihydroergot alkaloids also show a fluorescence decrease on irradiation, even though this group has already a saturated bond in the 9–10 position.

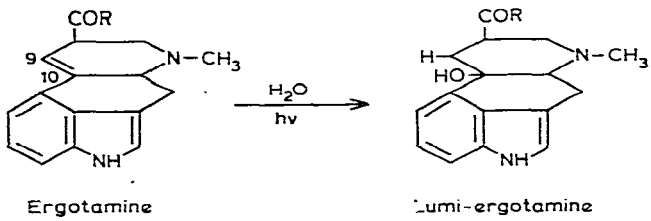

Fig. 1. A possible photochemical reaction of ergot alkaloids.

EXPERIMENTAL

Reagents

All ergot alkaloids were obtained from Sandoz (Basle, Switzerland). The water was doubly distilled. The acetonitrile was HPLC grade (Rathbury, Walkerburn, Great Britain) and the sodium hydrogen carbonate was analytical grade (Merck, Darmstadt, G.F.R.).

Instrumental

All experiments were carried out with the HPLC system described in Fig. 2

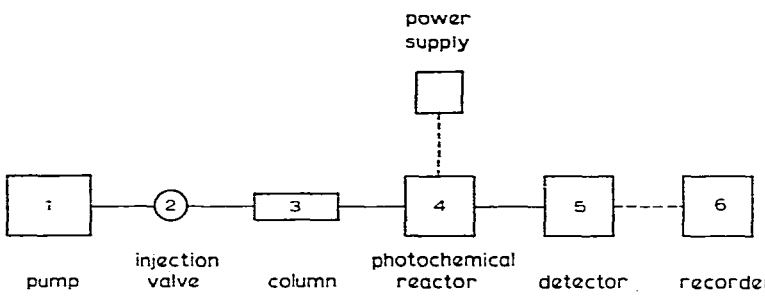

Fig. 2. Instrumental setup for HPLC with a photochemical reaction detector. 1, Waters M-6000 pump (Waters Assoc., Milford, Mass., U.S.A.); 2, Valco injection system (Valco, Houston, Texas, U.S.A.) with a 100-μl loop; 3, pre-packed column RP-18, 5-μm particle size (Knauer, Oberursel, Switzerland) 120 mm × 4.6 mm I.D.; 4, photochemical reactor; 5, spectrofluorometer SFM-22 (Kontron, Zurich, Switzerland) with a 50-μl flow-cell and operating at λ_{ex} = 327 nm and λ_{em} = 410 nm, for the dihydroergot alkaloids at λ_{ex} = 280 nm and λ_{em} = 340 nm; 6, recorder W + W 1100 (Kontron). Peaks were integrated with the Hewlett-Packard Laboratory Data System HF 3354.

Photochemical reactor

The reactor (Fig. 3) consists of a lamphouse and power supply (Siemens, 5 NS 1102) with a XBO 150W/1 Xenon high-pressure lamp. Around this lamp a coiled quartz capillary (1 m × 0.5 mm I.D.) (Felber, Basle, Switzerland) with a coil diameter of 6 cm is placed. Under the lamphouse, aluminium foil enhances the reflection of the emitted light. Cooling is achieved with pressurized air, precooled by solid CO_2 in a Dewar vessel.

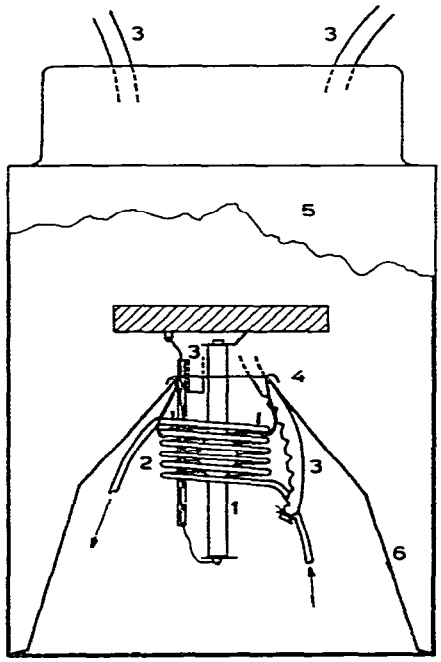

Fig. 3. The photochemical reactor. 1, Xenon high pressure lamp; 2, quartz capillary; 3, cooling; 4, clip; 5, lamphouse; 6, aluminium reflection shield.

RESULTS AND DISCUSSION

Band broadening in the reactor

The band broadening in the reactor, σ_v, when measured on non-irradiated peaks (with k' 1–3) was *ca.* 200 μl at a flow-rate of 1.5 ml/min. The band broadening could be reduced by fitting the quartz capillary with connectors of zero dead volume leading to the column and the detector, instead of PTFE and silicone tubes used in our experiments. An even smaller band broadening in the system can also be realized by using flow-segmentation techniques[4].

Effect of different parameters on the photochemical reaction

Influence of the flow. The lower the flow-rate, φ, the longer is the reaction time t_r (for $\varphi = 0.5$ ml/min $t_r = 53$ sec; for $\varphi = 1.0$ ml/min $t_r = 27$ sec; for $\varphi = 1.5$ ml/min $t_r = 18$ sec, *etc.*). Fig. 4 shows the influence of flow variation on the signal decrease, S_{irr}/S, where S_{irr} is the signal of an irradiated compound (peak areas or peak height) and S the signal of the non-irradiated compound. For positive identification of compounds, very low S_{irr}/S values, obtained at low flow-rates, are especially important. The flow variation has the same effect on the fluorescence signal of the 9,10-dihydroergot alkaloids.

Variation of concentration. The influence of the concentration on the signal decrease of five ergot alkaloids is shown in Fig. 5: the lower the concentration, the larger the decrease. At concentrations of *ca.* 10 ng/100 μl injection a plateau is reached, which means that the signal decrease is no longer dependent on the concentration. The same effect is observed with the dihydroergot alkaloids.

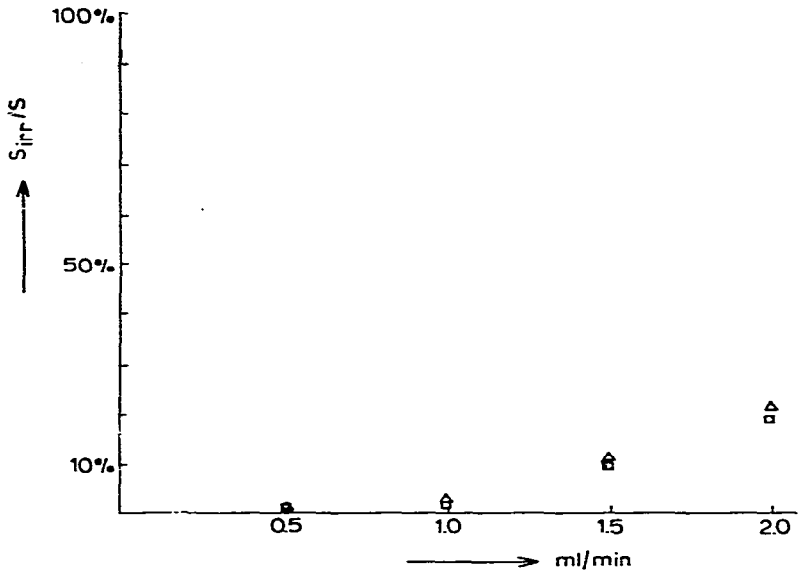

Fig. 4. The dependence of the signal decrease, S_{irr}/S, on the flow-rate. Conditions: eluent, 0.01 *M* $NcHCO_3$-acetonitrile (58:42, v/v) pH 2.1; sensitivity control medium, fine 50. Aci-ergotamine (0.14 μg/ml); aci-ergotaminine (0.19 μg/ml); ergotamine (0.19 μg/ml); ergocornine (0.19 μg/ml); and ergokryptine (0.23 μg/ml). △, Aci-ergotamine; □, other compounds.

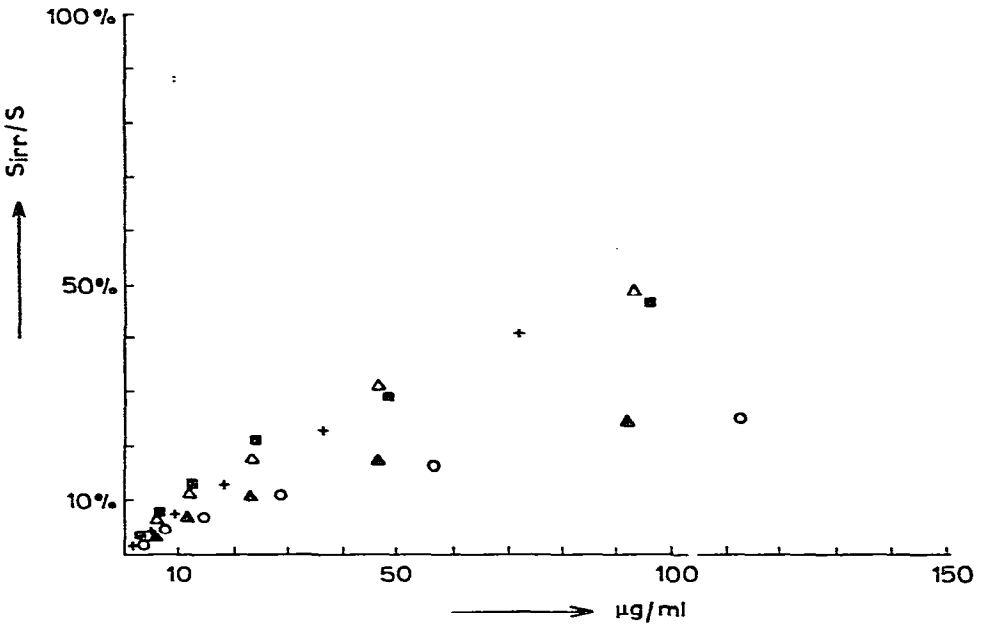

Fig. 5. Dependence of the signal decrease of five ergot alkaloids on their concentrations. Conditions: eluent 0.01 *M* $NaHCO_3$–acetonitrile (58:42, v/v); pH 8.5; sensitivity control low, fine 50; flow-rate 1.5 ml/min. △, Aci-ergotamine; ■, ergotamine; +, aci-ergotaminine; ▲, ergocornine; ○, ergokryptine.

Influence of the composition of the mobile phase. Changing the percentage of acetonitrile in the eluent by 4%, from 58:42 (v/v) to 62:38 (v/v) 0.01 *M* $NaHCO_3$–acetonitrile at pH 8.5, results in an increase of k' values by *ca.* 100% (see Table I); the signal decrease S_{irr}/S also becomes higher. The increase of the S_{irr}/S value at higher percentages of acetonitrile is due to a higher value of S_{irr}, resulting from the lower polarity of acetonitrile in the mobile phase. The same effect is noticeable in batch experiments with dihydroergotamine.

TABLE I

SIGNAL RATIOS (S_{irr}/S) AND RETENTION DATA OF VARIOUS ALKALOIDS AS A FUNCTION OF DIFFERENT ELUENT COMPOSITIONS

*Eluent**	*pH*	*Compound***	*k'*	*Concentration**** *(μg/ml)*	*Measured* S_{irr}/S
58:42 (v/v)	8.5	A-E	0.8	23.3	0.314
		A-Erm	2.3	11.2	0.230
		E	2.9	11.1	0.291
		E-C	4.6	8.4	0.175
		E-K	6.5	8.4	0.170
62:38 (v/v)	8.5	A-E	1.5	16.8	0.344
		A-Erm	4.4	6.9	0.196
		E	5.5	6.7	0.277
		E-C	8.7	4.9	0.169
		E-K	12.6	4.6	0.171
58:42 (v/v)	2.2	A-E	1.2	12.8	0.483
		A-Erm	1.6	8.9	0.487
		E	1.8	11.7	0.521
		E-C	2.2	9.7	0.459
		E-K	3.0	9.4	0.475
62:38 (v/v)	2.2	A-E	1.3	12.7	0.383
		A-Erm	1.8	8.2	0.396
		E	2.3	10.1	0.422
		E-C	2.7	8.2	0.396
		E-K	3.9	7.7	0.376

* 0.01 *M* $NaHCO_3$–acetonitrile.

** A-E = aci-ergotamine; A-Erm = aci-ergotaminine; E = ergotamine; E-C = ergocornine; E-K = ergokryptine.

*** For correction of these values, see eqn. 1.

An increase in absolute signal of *ca.* 300% was observed when the percentage of acetonitrile was varied from 30 to 50%. It was noticeable that the measured S_{irr}/S values were lower for compounds with a higher k'. This could be due to dilution in the column and the reactor. The concentrations in Table I are therefore corrected for this effect according to eqn. 1:

$$\frac{C_{max}}{C_0} = \frac{V_0}{AL\sqrt{2\pi^3}} \cdot \frac{N^{1/2}}{(1 + k')} \quad (1)$$

where C_{max} is the concentration at the peak maximum, C_0 the injected concentration,

V_0 the injection volume, A the cross-section of the separation column, L the length of the column, N the plate number and k' the capacity factor. When the percentage of acetonitrile at pH 8.5 was changed, ergokryptine, for instance, gives the same S_{irr}/S value for two very different concentrations (see Table I). Plotting S_{irr}/S as a function of these corrected concentrations, we found that the decrease in fluorescence (S_{irr}/S) was almost the same for all the measured compounds.

A drop of the pH in the mobile phase from 8.5 to 2.2 produced a pronounced change in the k' values, which can be attributed to the ionic form in which the alkaloids exist at this low pH. After correction again for dilution in the column and the reactor, it becomes obvious that the S_{irr}/S values in an acidic medium are much higher. Possibly the 9–10 position in the molecule is stabilized and attack by a H_3O^+ ion becomes more difficult. An increase in the signal of *ca.* 30% was observed when the pH was lowered to 1.5 in batch experiments with dihydroergotamine at eluent compositions of 30–50% acetonitrile.

Effect of irradiation on band broadening

An interesting observation is that band broadening in the reactor is substantially reduced following irradiation. This is due to the fact that at lower ergot alkaloid concentrations a greater signal decrease is obtained. The improved resolution after irradiation is seen in Fig. 6. This is typical for this ergot system. The following

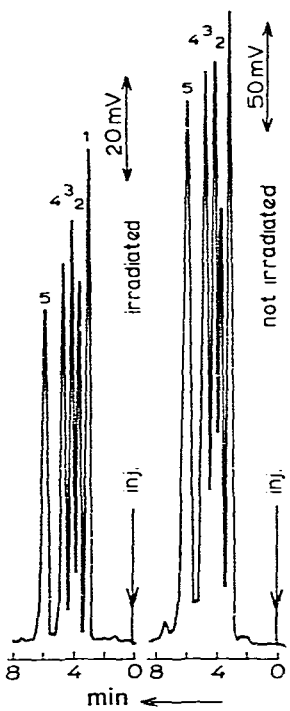

Fig. 6. Separation of five ergot alkaloids. Conditions: eluent, 0.01 *M* $NaHCO_3$–acetonitrile (58:42, v/v); sensitivity control low, fine 50; flow-rate 1.5 ml/min. 1, aci-ergotamine (11.7 μg/ml); 2, aci-ergotaminine (9.1 μg/ml); 3, ergotamine (12.0 μg/ml); 4, ergocornine (11.6 μg/ml); 5, ergokryptine (14.2 μg/ml).

explanation can be given. Let us assume that the peaks, entering the detector, are approximately Gaussian:

$$C = C_{max} \exp [-(t - t_R)^2/2\sigma^2] \quad (2)$$

and the concentration dependence of S_{irr}/S is expressed as

$$S_{irr}/S = a \times c + b \quad (3)$$

with $a = 0.008$ and $b = 0.08$ for aci-ergotamine, where a and b were calculated from experimental data shown in Fig. 5. It can be seen that the combination of eqns. 2 and 3 leads to smaller σ values for irradiated peaks. This effect is shown in Fig. 7.

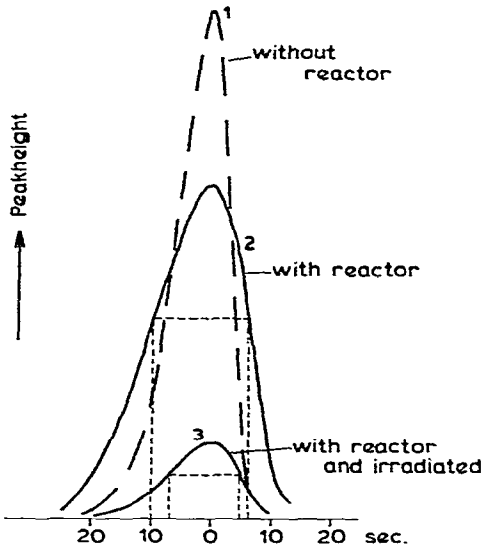

Fig. 7. The influence of reactor and irradiation on peak shape. Compound, aci-ergotamine; eluent, 0.01 M $NaHCO_3$–acetonitrile (58:42, v/v); pH 8.5, flow-rate 1.5 ml/min. 1, Without reactor (2σ = 9 sec); 2, with reactor (2σ = 16 sec); 3, with reactor and irradiated (2σ = 11.5 sec).

Selective identification of ergot alkaloids

For selective identification of ergot alkaloids a decrease of at least 90% in the fluorescence signal is necessary. This is best achieved with a low flow-rate (*e.g.* 0.5 ml/min), a low percentage of acetonitrile and a high pH. However, the amount of acetonitrile and the pH strongly influence the separation (see Table I). Therefore the possible variations are limited. The use of more polar chemically bonded surfaces might improve this situation. The use of a high pH shortens the column life and decreases its separation power[5]. Therefore the following conditions represent a good compromise: 0.01 M $NaHCO_3$–acetonitrile (58:42, v/v) at pH 2.2 at a flow-rate of 0.5 ml/min. Under these conditions the fluorescence decrease was 90–99% for 17 ergot alkaloids and dihydroergot alkaloids.

In a spiked urine sample one can see the decrease of the signals of dihydroergotoxine (Fig. 8) and various other ergot alkaloids in relation to a blank urine (Fig. 9).

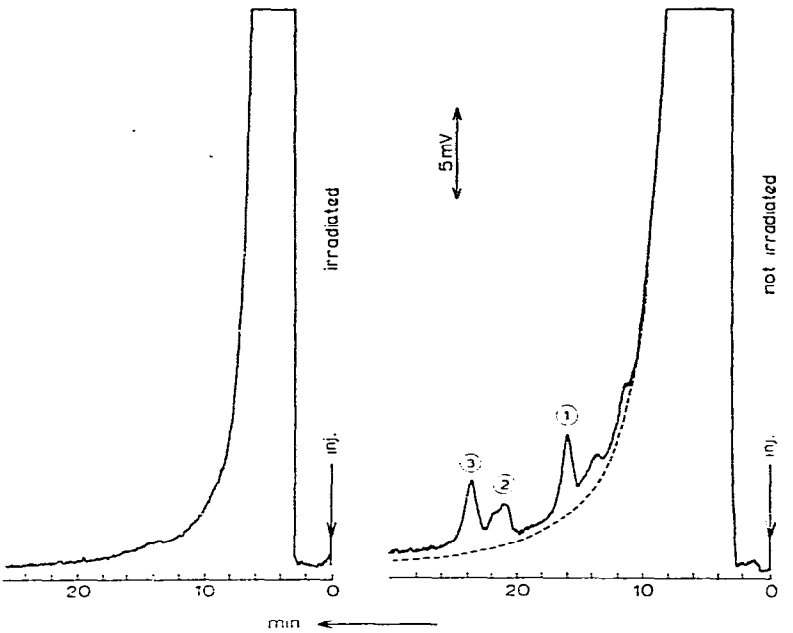

Fig. 8. Urine sample, spiked with dihydroergotoxine. $\lambda_{em} = 280$ nm, $\lambda_{em} = 340$ nm; sensitivity control medium, fine 50; flow-rate 0.5 ml/min; $v_p = 30$ cm/h; recorder 5 mV; inj. 100 μl. 1, Dihydroergocornine (46 ng/ml); 2, $\alpha + \beta$ dihydroergokryptine (46 ng/ml); 3, dihydroergocristine (46 ng/ml). – – –, blank urine.

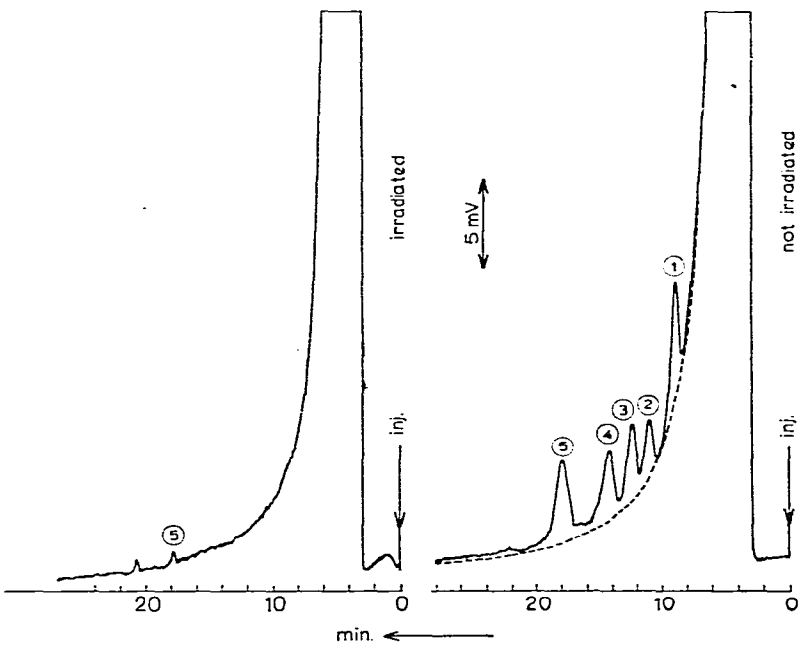

Fig. 9. Urine sample, spiked with 5 ergot alkaloids. $\lambda_{ex} = 327$ nm, $\lambda_{em} = 415$ nm; sensitivity control medium, fine 50; $v_p = 30$ cm/h; recorder 5 mV; inj. 100 μl. 1, Aci-ergotamine; 2, aci-ergotaminine; 3, ergotamine; 4, ergocornine; 5, ergokryptine; each 0.2 μg/ml. – – –, blank urine.

CONCLUSIONS

The photochemical detection technique in HPLC offers a selective identification for all the ergot alkaloids tested. Although it is suspected that the mechanism in Fig. 1 is not necessarily followed under the high-density irradiation conditions used here, one can conclude that the technique is of general applicability to ergot alkaloids. Preliminary results[6] suggest that the drastic fluorescence decrease may be at least partly due to a change in the indole ring, since indole exhibits the same behaviour on irradiation. The technique is suitable for qualitative work in complex samples.

The present study also indicates some of the general possibilities and the potential use of photochemical reactions for post-column reaction detectors in HPLC.

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