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Stability-indicating high-performance liquid chromatographic assay and analysis of decomposition products of 2-{4-[(7-chloro-2-quinoxalinyl)oxy]phenoxy}propanoic acid

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Abstract

A stability-indicating HPLC assay has been developed for 2-{4-[(7-chloro-2-quinoxalinyl)oxy]phenoxy}propanoic acid (XK469). XK-469 is the 7-chloro analog of herbicide Quizalofop and is currently under development as an antineoplastic agent. HPLC separation of XK469 is achieved with an ODS column using isocratic elution of an aqueous MeOH mobile phase. The assay is reproducible (RSD=0.9%), linear (r^2=0.999), accurate (error=1.2%) and sensitive (LDL=1.2 ng). The HPLC separates XK469 from its forced decomposition products. Identities of the decomposition products have been elucidated.

Keywords: Pharmaceutical analysis; Chloroquinoxalinyloxyphenoxypropanoic acid; Aryloxyphenoxypropanoic acid

1. Introduction

Aryloxyphenoxypropanoates have been developed and used as herbicides [1–5]. Several 2-[(quinoxalinyoxy)phenoxy]propanoates have also been targeted as anticancer agents [6]. One of them, 2-{4-[(7-chloro-2-quinoxalinyl)oxy]phenoxy}propanoic acid (XK469), the 7-chloro analog of the herbicide Quizalofop, is being investigated by the National Cancer Institute as an antineoplastic agent. Gas chromatography (GC) [7,8] and high-performance liquid chromatography (HPLC) [9–12] methods have appeared in literature for Quizalofop, none has been reported for XK469. The reported methods are for residual determination of herbicides in soil and water samples. The GC methods require derivatization. None of the reported methods are stability-indicating for the herbicides, however. Because of current pharmaceutical interest, a simple stability-indicating assay for native XK469 is warranted. This paper presents a reversed-phase HPLC assay for XK469. The assay has been validated for stability-indicating, repeatability, linearity and accuracy. Forced decomposition products of XK469 were formed and identified with their UV and mass spectral data.

2. Experimental

2.1. Reagents and materials

Anhydrous trifluoroacetic acid (TFA) was from Sigma (St. Louis, MO, USA). HPLC-grade methanol

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(MeOH) was purchased from Mallinckrodt (Paris, KY, USA). Solutions of 0.1 *M* HCl and NaOH were prepared from Dilute-it Analytical Concentrate (J.T. Baker, Phillipsburg, NJ, USA). Acid and base solutions were prepared with water purified through a Millipore Super-Q pure Water System (Waltham, MA, USA).

XK469 samples were provided by the National Cancer Institute (Bethesda, MD, USA). Phenanthrene, the internal standard, was obtained from Eastman (Rochester, NY, USA). The internal standard solution (ISS) was prepared by dissolving 4 mg phenanthrene per 100 ml MeOH. Unless otherwise mentioned, sample test solutions for assay validation were prepared in the ISS to yield 0.08–0.3 mg ml^{-1} of XK469.

2.2. Forced XK 469 decomposition

A stock solution of XK469 was prepared in MeOH at a concentration of 1 mg ml^{-1}. Aliquots of the stock were individually diluted with equal volumes of MeOH, 0.1 *M* HCl or 0.1 *M* NaOH to form sample solutions S1, S2 or S3, respectively. Portions of each S1, S2 and S3 were heated in a H2025 Temp Blok Module Heater (Scientific Products Division of American Hospital Supply, McGaw Park, IL, USA) for 2 h at 70°C to create forced decomposition

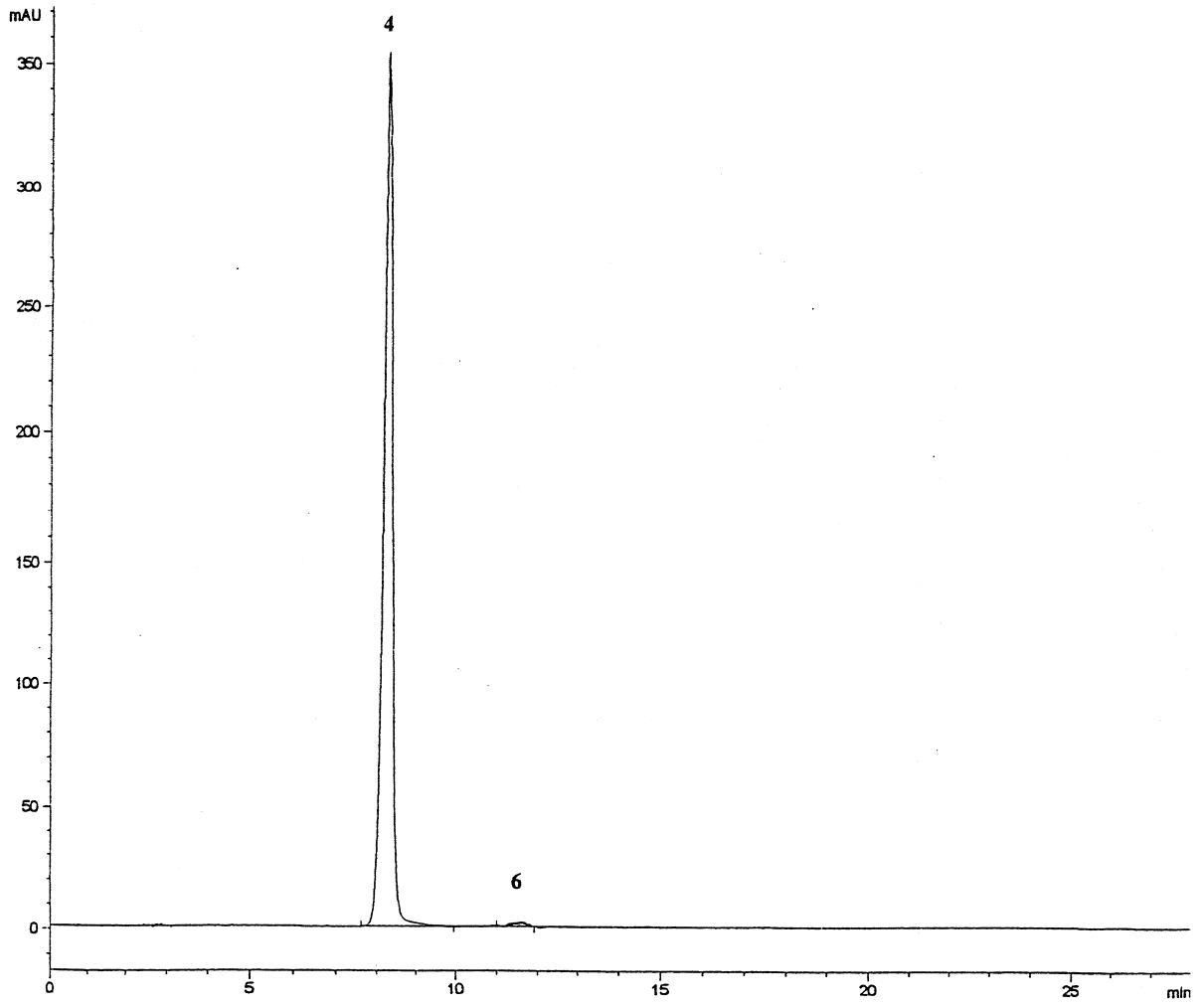

Fig. 1. HPLC chromatogram of a fresh solution of XK469 in MeOH (S1). See text for experimental details.

products. A small amount (1 mg) of bulk XK469 was similarly heated for 24 h, then dissolved in 2.0 ml MeOH to generate S4.

2.3. HPLC

The HPLC assay system consisted of a Varian (Walnut Creek, CA, USA) Vista 5500 HPLC pump, a Thermoseparation (Fremont, CA, USA) AS3000 Autosampler, and a Perkin-Elmer (Norfolk, CT, USA) LC135 UV detector. Detection wavelength of the detector was set at 255 and 245 nm, since it can only be set at increments of 5 nm. For monitoring the UV profiles of forced decomposition products, the detector was switched to a Hewlett-Packard (HP) (Wilmington, DE, USA) 1090 photodiode array detection (DAD) system. Data were collected and processed with the HP 3D ChemStation. Sample and test solutions (10 μl) were loaded on an Alltech (Deerfield, IL, USA) Adsorbosphere-HS C_{18}, 5 μm, 250×4.6 mm I.D. stainless steel column. Chromatographic analysis was carried out by isocratic elution with a mixture of MeOH–water (80:20) containing 0.1% TFA, at 1 ml min^{-1}. LC–MS was performed with a Scientific Systems (StateCollege, PA, USA) Model 222/232 HPLC pump system equipped with a Vestec (Houston, TX, USA) LC Thermospray interface and a 201 XL mass spectrometer. For LC–MS, 0.1 *M* ammonium acetate was substituted for 0.1% TFA in the mobile phase.

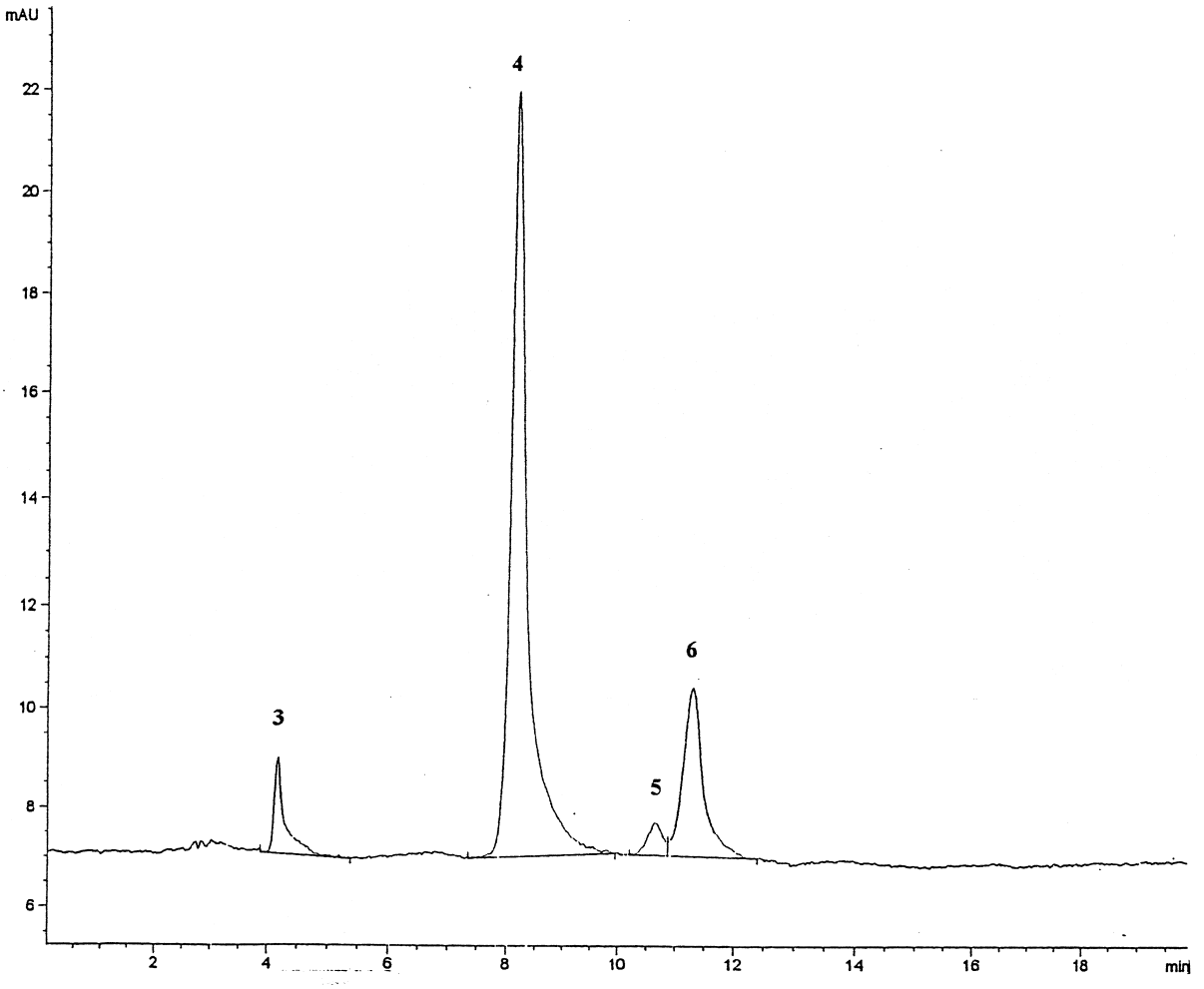

Fig. 2. HPLC chromatogram of a heated solution of XK469 in MeOH–0.1 *M* HCl (S2). See text for experimental details.

3. Results

3.1. HPLC separation and identity of decomposition products

HPLC chromatograms of S2, S3 and S4 are identical to those of the fresh and heated S1. Fig. 1 presents the chromatogram of the fresh S1. It shows XK469 contains a minor impurity (**6**, 11.3 min). Heating the acidified solution S2 enhanced the impurity (Fig. 2). In addition, significant amounts of **3** (4.2 min) and **5** (10.7 min) were also formed. The former, **3**, is the major product generated in the heated basified solution S3 (Fig. 3). Heating S3 also gave rise to minor products (**1**, 2.8 min; **2**, 3.0 min) at the unretained area. The impurities and decomposition products were well resolved from XK469. DAD spectral analysis verified the homogeneity of the XK469 peaks (**4**) in all solutions.

The decomposition products were all generated under solvolytic conditions. Thus, solvolysis products B–F depicted in Fig. 4 are their possible identities. XK469 (A, Fig. 4) contains two non-conjugated chromophores, quinoxaline and 1,4-dihydroxybenzene (hydroquinone). Quinoxaline has a strong band at 235 nm and a weaker one at 320 nm [13]. The 320 nm band is believed to be the composite of both L_a and L_b bands which are

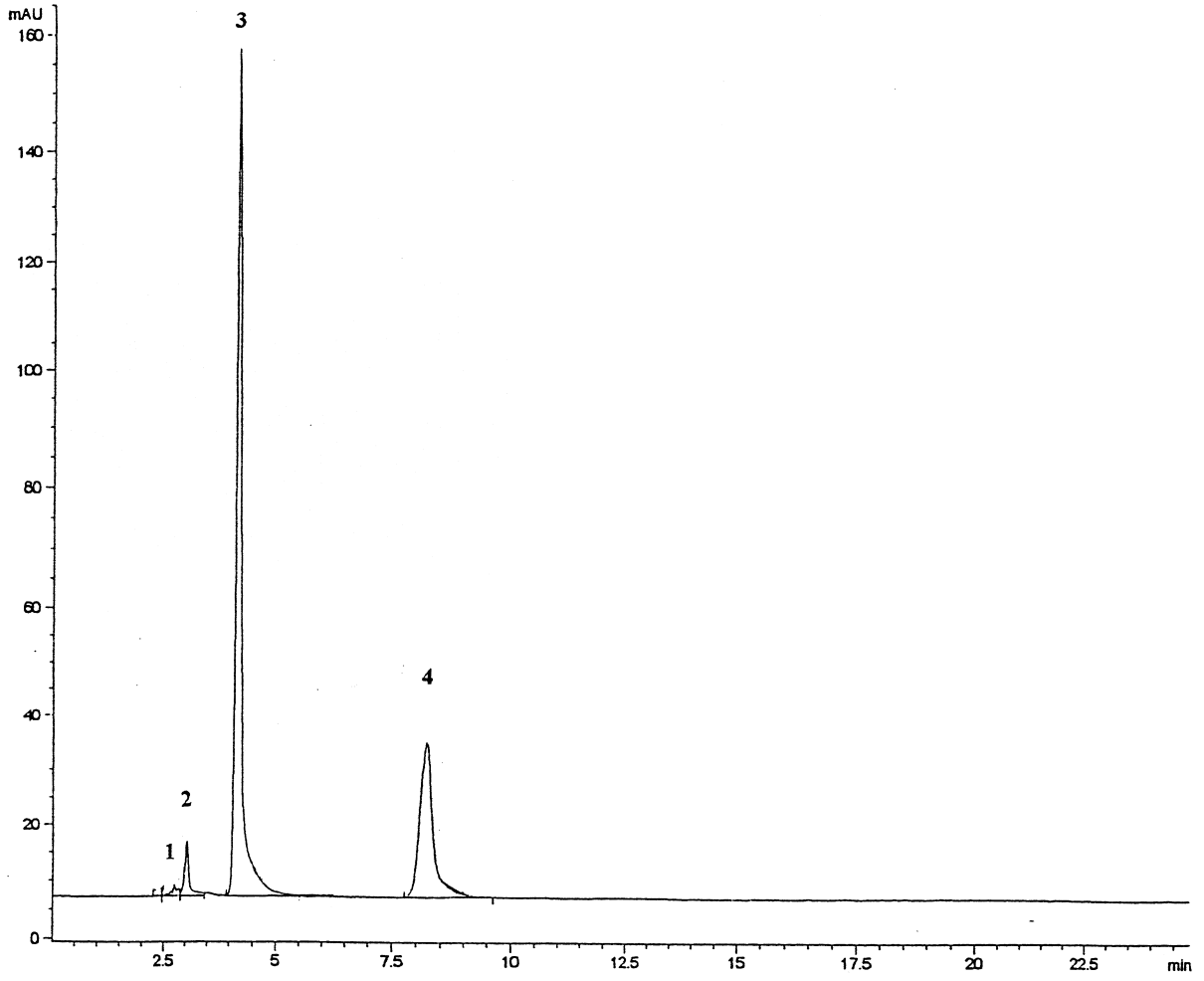

Fig. 3. HPLC chromatogram of a heated solution of XK469 in MeOH–0.1 *M* NaOH (S3). See text for experimental details.

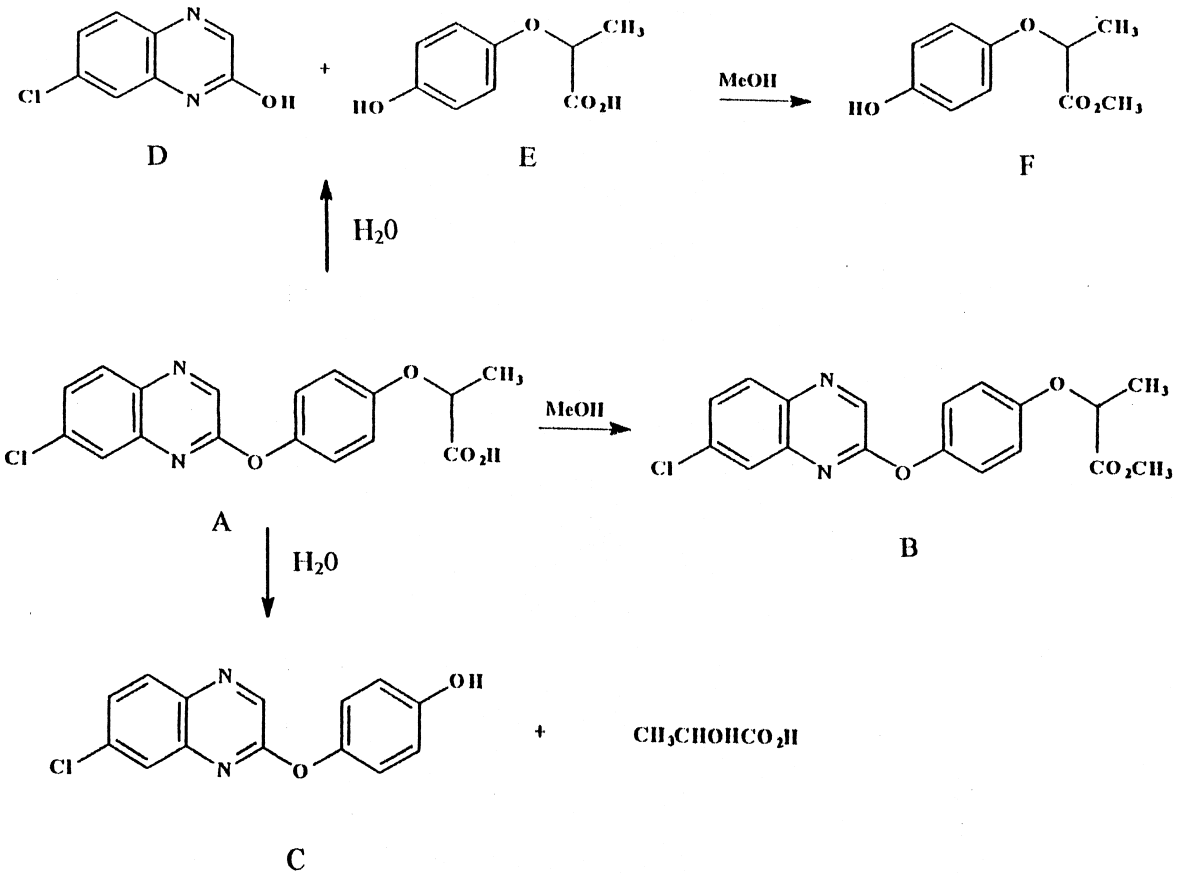

Fig. 4. Solvolytic pathways of XK469 in aqueous MeOH.

normally at 270 and 310 nm, respectively, for diaza-substituted naphthalenes (e.g., quinazoline) [13]. Hydroquinone has a single absorption maximum at 283 nm or at 295 nm if mono-ionized [14]. Therefore, solvolysis products of XK469 may have UV spectra different from that of the parent. UV spectra of the decomposition products can be grouped into three types as presented in Fig. 5. Those of products **5** and **6,** identical to that of XK469 (**4),** showing maxima at 245, 276 and 320–340 nm (Fig. 5a). Structures of **5** and **6** must differ from XK469 only in the propanoic acid moiety. They are probably B and C (Fig. 4). Product **3**, whose UV profile (Fig. 5b) is slightly different from that of the parent (Fig. 5a), still possesses the quinoxaline ring and could possibly be D. UV profiles of **1** and **2** (Fig. 5c) are different and much simpler than that of XK469. In fact, they are similar to that of mono-ionized hydroquinone. Their lack of absorption maximum at 320–340 nm suggest that **1** and **2** are void of the quinoxaline moiety and are likely compounds E and F.

Fig. 6 presents the LC–MS of **1–6** which confirms the identities of products **2–6**. **1** and **3** were detected as negative ions while the rest were detected in the normal positive mode. MS of **1** and **2** (Fig. 6a and b), suggesting molecular mass (M_r) of 182 and 196, are consistent with the free acid (E) and methyl ester (F) of 2-(4-hydroxyphenoxy)propanoic acid, respectively. MS of **3** (Fig. 6c), indicating a M_r of 180/182 (3:1), is congruent with 2-hydroxy-7-chloroquinoxaline (D). The M_r of 272/274 for **5** (Fig. 6e) is in agreement with 4-(7-chloroquinoxalinyloxy)phenol (C). That of **6** (Fig. 6f) is 14 more than the M_r of XK469 (Fig. 6d) and is a clear indication that **6** is the methyl ester of XK469 (B). Identities of the

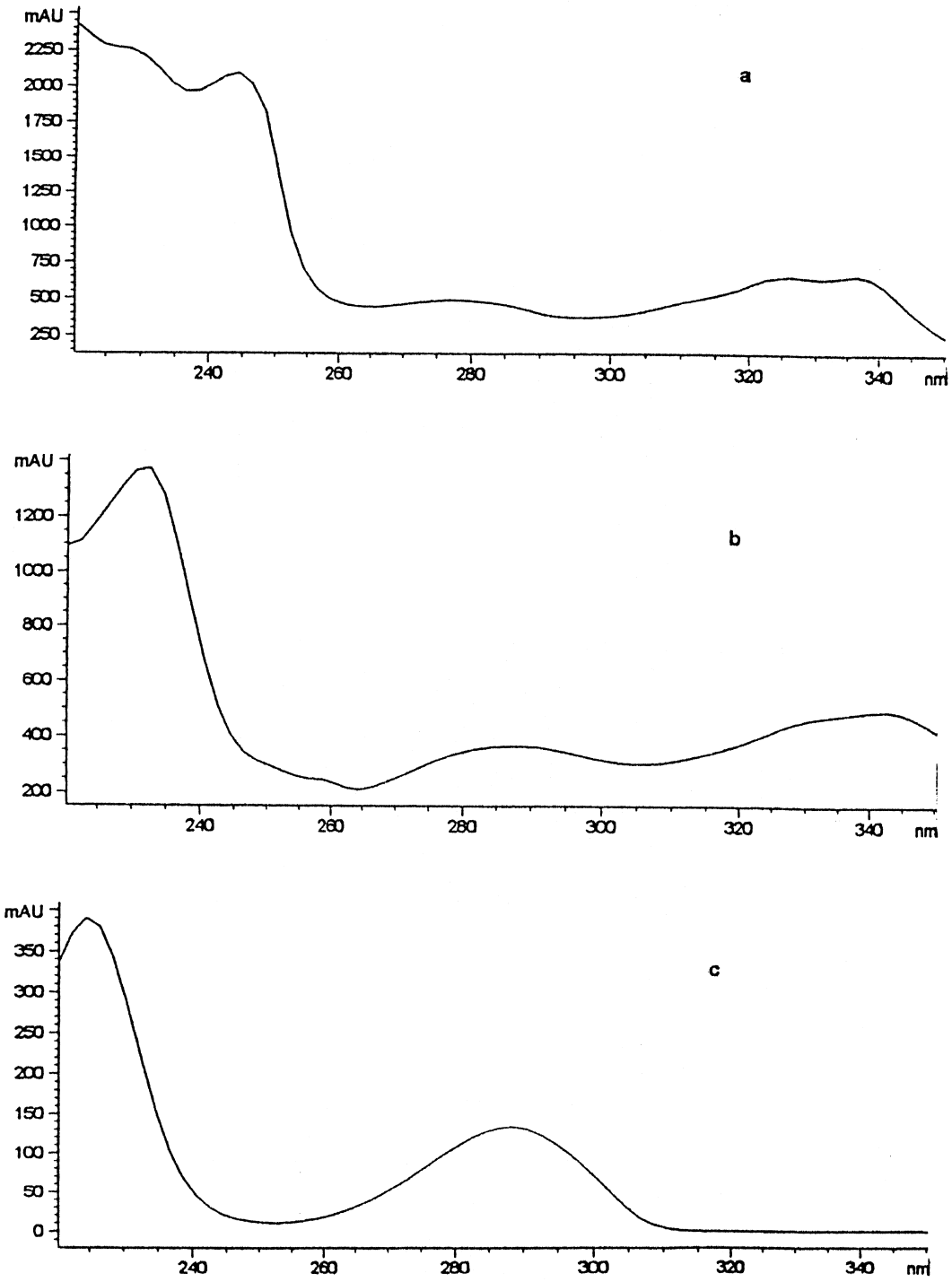

Fig. 5. UV profiles of the HPLC peaks for XK469 and its solvolytic products: (a) **4**, **5**, **6**; (b) **3**; and (c) **1**, **2**.

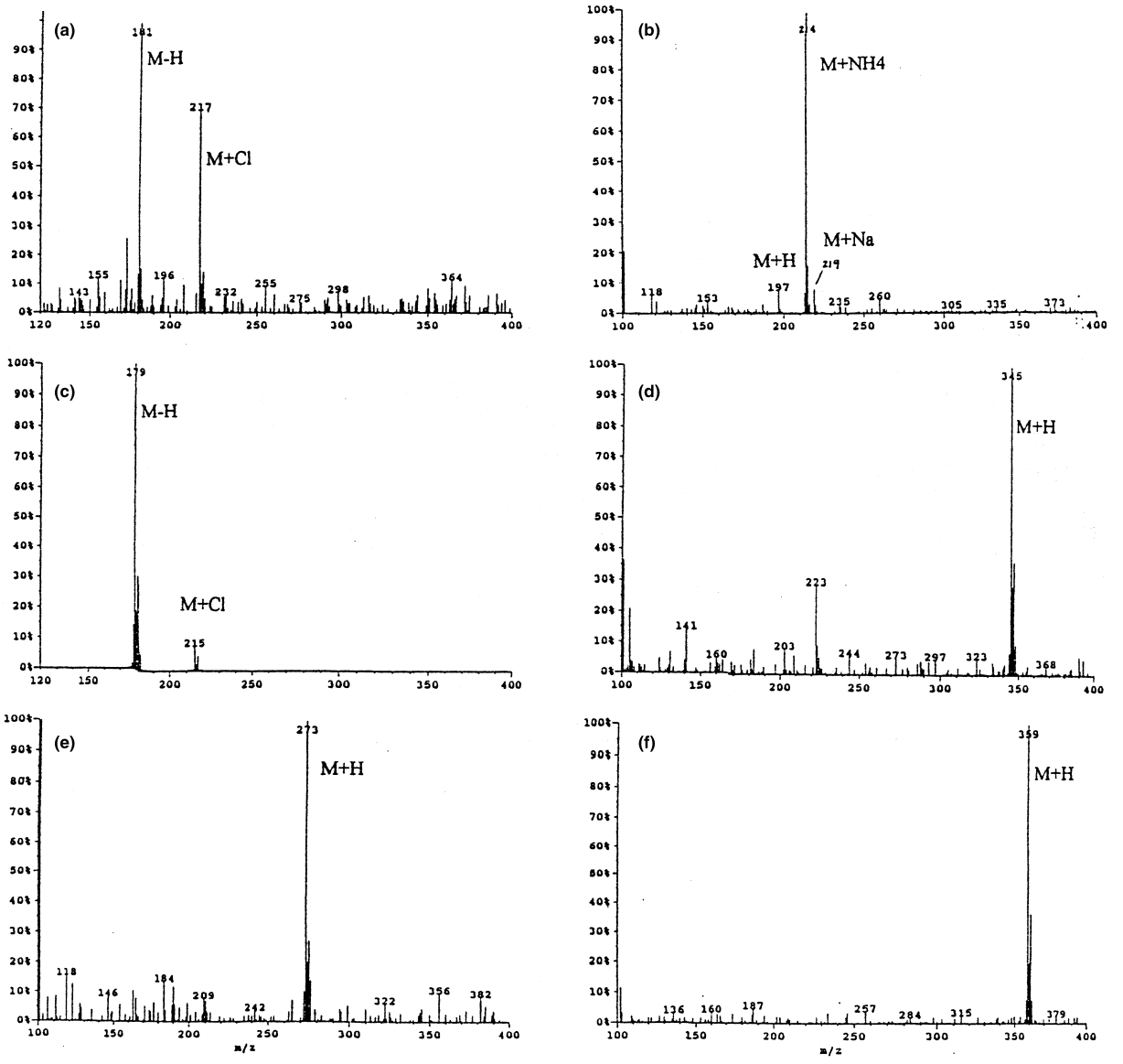

Fig. 6. Mass spectra of the HPLC peaks for XK469 and its solvolytic products: (a) **1**, (b) **2**, (c) **3**, (d) **4**, (e) **5**, and (f) **6**.

HPLC peaks and their spectral data are summarized in Table 1.

3.2. HPLC assay validation

The pK_a of XK469 was potentiometrically determined as 3.6 which is in agreement with literature values (3.1–3.2) for chlorophenoxyacetic acids [15]. However, dissolution of XK469 in aqueous solvents turns out to be difficult and is a potential problem for HPLC assay. Solubility of XK469 in 0.1 *M* NaOH (>30 mg ml^{-1}) and in pH 8 phosphate and hydrogencarbonate buffers (>10 mg ml^{-1}) are good, but more than 15 min of vigorous vortexing or sonication is required. Otherwise, <0.5 mg ml^{-1} was achieved with the pH 8 buffers if normal vigorous shaking with intermittently brief sonication were used. When the NaOH solution was diluted with

Table 1
Retention (k') and spectral data, and identities of HPLC peaks in Figs. 1–3

Peak	k'	MS data	UV	Identity
1	0.12	181 (M−H)	Fig. 5c	2-(4-Hydroxyphenoxy)propanoic acid (E)
2	0.20	197 (M+H)	Fig. 5c	Methyl ester of C (F)
3	0.68	179 (M−H)	Fig. 5b	2-Hydroxy-7-chloro-quinoxaline (D)
4	2.24	345 (M+H)	Fig. 5a	XK469 (A)
5	3.28	273 (M+H)	Fig. 5a	4-(7-Chloro-2-quinoxalinyloxy)phenol (C)
6	3.52	359 (M+H)	Fig. 5a	Methyl ester of XK469 (B)

phosphate buffer to a final pH of 7, the solution was saturated at considerably less than 10 mg ml^{-1}. Long term observation of the supernatant revealed continuous decrease in concentration. Filtration experiments suggest formation of molecular aggregates or micelles. Because of the difficulty in solubilizing and keeping the compound in aqueous solutions, sample solutions of XK469 should be dissolved in MeOH whenever possible for HPLC assay.

Fig. 7 is a typical chromatogram of the HPLC

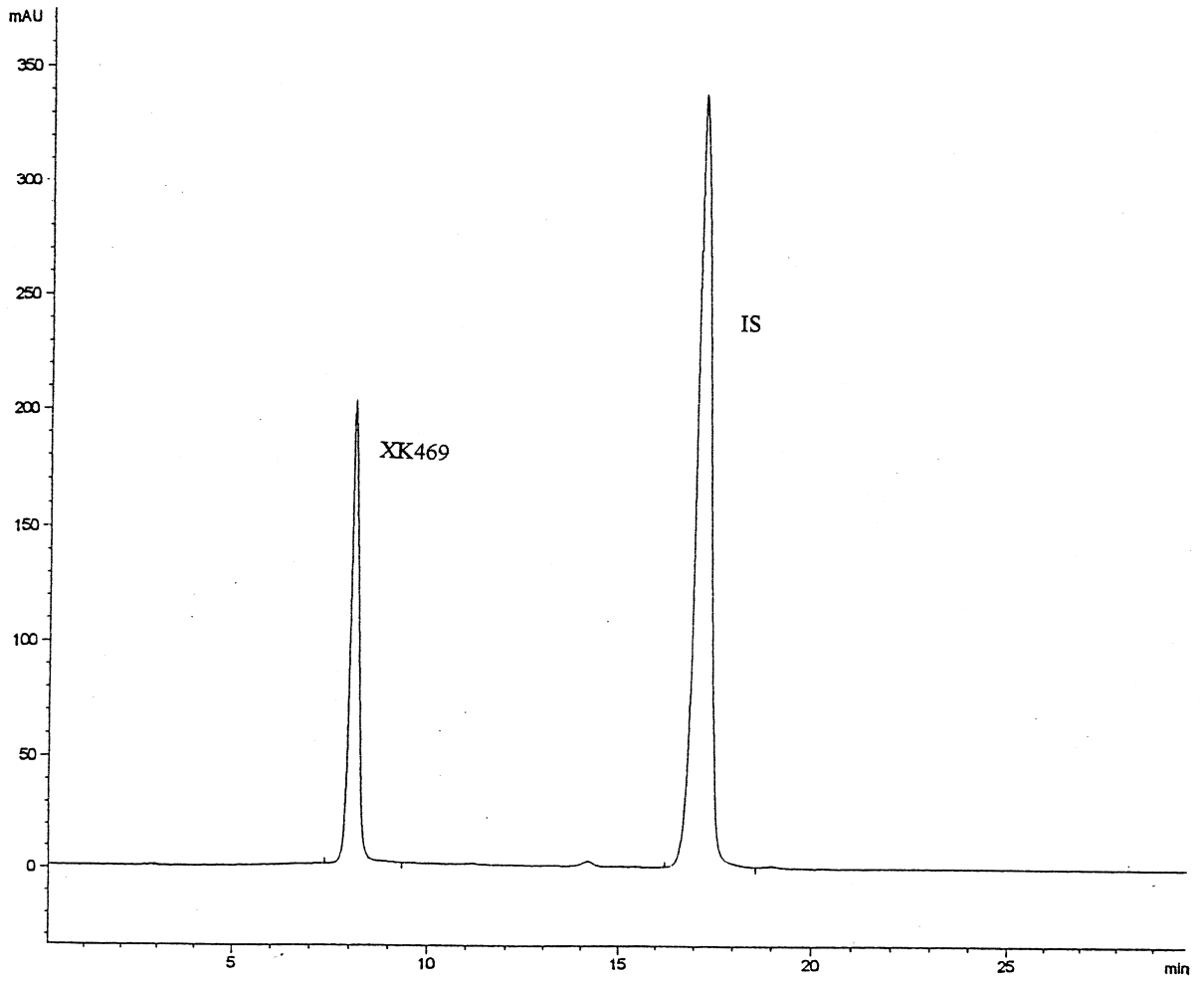

Fig. 7. Typical HPLC chromatogram of a test solution for assay validation. See text for experimental details.

Table 2
Linearity and accuracy data for HPLC validation of XK469 assay[a]

Standard	Peak intensity			mg ml^{-1} of XK469 (XK)		Error[c] (%)
	XK	I.S.	F=XK/I.S.	Actual	Found[b]	
1	1071	4313	0.248	0.0818	0.0796	−2.7
2	1306	4328	0.302	0.0973	0.0968	−0.5
3	2116	4326	0.489	0.1561	0.1572	+0.7
4	2597	4291	0.605	0.1925	0.1946	+1.1
5	3059	4324	0.707	0.2249	0.2275	+1.2
6	3850	4350	0.885	0.2881	0.2847	−1.2
					Average[d]	1.2

[a] See text for HPLC conditions. Linear regression analysis of F (x) vs. actual XK concentration (y) gave $y=0.3104x+0.0011$, $r^2=0.9991$. Standard errors of slope and intercept were 0.0048 and 0.0084, respectively.
[b] Found XK concentration=(F−0.0011)/0.3104.
[c] Error=(found XK conc.−actual XK conc.)/actual XK conc.
[d] Average=Σ|error|/6.

assay. The 17 min peak is the internal standard, phenanthrene, which is well resolved from XK469 and its potential products. Validation of the HPLC assay was based on chromatographic data derived from XK469 standard solutions. The repeatability (RSD) obtained from six different standard solutions of similar concentration (140–160 μg ml^{-1} of XK469 in the ISS solution) was 0.9% within-day and 0.8% between-days ($n=3$). Based on data from five standard solutions (Table 2), the assay was linear ($r^2=0.9991$, slope=0.3105 with standard error of 0.0048, and intercept=0.0011 with standard error of 0.0084) and accurate (error=1.2%). Based on a 3:1 signal-to-noise ratio, the lower detection limit (LDL) for XK469 was 1.5 ng. Validation results at 245 nm, the absorption maximum of XK469 were very similar to those at 255 nm. The repeatability of the assay was 0.9% within-day ($n=4$) and 0.8% between-days ($n=3$). The assay was linear ($r^2=0.9992$, slope=0.5776 with standard error of 0.0097, intercept=0.0223 with standard error of 0.0167) except that the LDL was 0.5 ng. This is consistent with the UV absorptivity of XK469 at 245 nm which is three times more intense than at 255 nm (Fig. 5a). Accuracy of the assay was further demonstrated by the analysis of two lots of XK469 samples. Potency was established as 99.6% for lot HD1 and 99.4% for lot HD2 based material balance consideration of their elemental, HPLC impurity, moisture and organic volatile data. The potency results for lot HD2, using the validated HPLC assay and the 99.6% pure lot HD1 as the working standard, were 99.8% (at 245 nm detection) and 99.0% (at 255 nm detection) for an average of 99.4%. RSD of the assay was <1.0%, $n=4$. The assay results were well within the experimental error of that derived from mass balance consideration.

4. Conclusion

A rapid and direct determination of XK469, an aryoxyphenoxypropanoic acid has been developed and validated. The assay is stability-indicating. It resolves XK469 from potential decomposition products. Identities of forced decomposition products have been elucidated with their UV and mass spectral data.

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