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## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF UREA AND RELATED COMPOUNDS WITH POST-COLUMN DERIVATIZATION

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### SUMMARY

Urea and related compounds such as allantoin, N-methylurea, 1,1-dimethylurea, 1,3-dimethylurea, thiourea and thiourea dioxide can be determined within 30 min by high-performance liquid chromatography with a detection limit for each of less than 0.2  $\mu\text{g}$ .

The post-column detection system consists of a three-reagent delivery system. Urea and related compounds from an analytical column are converted into the corresponding N-chloramines with hypochlorite and the excess of hypochlorite is selectively destroyed with nitrite. The N-chloramines formed are reacted with iodide to form triiodide, which is monitored at 370 nm.

These compounds were separated with a strong cation exchanger (TSK-Gel LS-211, 12  $\mu\text{m}$ ) in the potassium, sodium and lithium forms packed in a glass column (400  $\times$  8 mm I.D.). Useful differences in retention and selectivity occur with these ionic forms and different column temperatures; the lithium form of the resin at 50°C is optimal with deionized water as the eluent.

The method has been applied successfully to the determination of the above compounds, which are widely used as additives in drugs, chelating agents, anticaking agents and bleaching agents.

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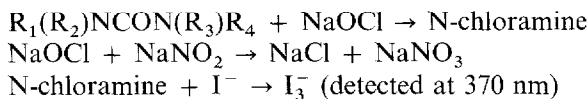
### INTRODUCTION

Allantoin, urea and the related compounds are widely used as drugs, chelating agents, reductive bleachers and caking inhibitors. Some of them are biologically important as purine oxidation products.

Previously, urea has been determined by colour and fluorescent reactions and enzymatic reactions. For allantoin, most of the determinations have been based on the detection of hydrolysed products, allantoic acid and/or glyoxylic acid. These methods need cumbersome sample preparation procedures for the elimination of the interferences present. Little has been reported on the analysis of the substituted compounds.

It is well known that the reaction of amines, ammonia and amides with hypochlorite produces N-chloramine derivatives under various conditions<sup>1</sup>. The reaction

of urea with hypochlorite, the so-called "Hoffmann degradation", which produces hydrazine, followed by condensation with salicylaldehyde to form fluorescent salicylaldazine, has been used in the qualitative trade analysis of urea<sup>2</sup>. It has been found that allantoin, urea and the related compounds can be converted into the corresponding N-chloramines with hypochlorite and can be detected by the following post-column reactions:



This paper describes a convenient and direct determination of allantoin, urea and related low-molecular-weight compounds by high-performance liquid chromatography with a selective post-column technique.

## EXPERIMENTAL

### Reagents

The low-molecular-weight urea derivatives used were allantoin, urea, N-methylurea, 1,1-dimethylurea, 1,3-dimethylurea, tetramethylurea, thiourea and thiourea dioxide (Wako, Osaka, Japan).

The recommended eluent was deionized water. The three post-column reagents used were as follows: hypochlorite reagent [0.25% (w/v) sodium hypochlorite in 0.5 M phosphate buffer (pH 4.5)]; nitrite reagent [0.025% (w/v) sodium nitrite in deionized water]; and iodide reagent [4% (w/v) potassium iodide in deionized water].

### Apparatus and procedure

Fig. 1 is a schematic diagram of the liquid chromatograph. Pump 1 (Model NP-50, Nihon Seimitsu, Tokyo, Japan) was used to pump the eluent (2) at a flow-rate of 1.0 ml/min. Sample solutions were injected via a sampling valve (3) (Hitachi Model 056) and the sample compounds were separated with a strong cation exchanger (TSK-Gel LS-211, Li<sup>+</sup>, 12 μm) packed in a glass column 400 × 8 mm I.D. (4) by a slurry packing procedure. The column temperature was maintained at 50°C by a water-bath (5). Three acid-resistant pumps (6–8) (Nihon Seimitsu) were used to pump

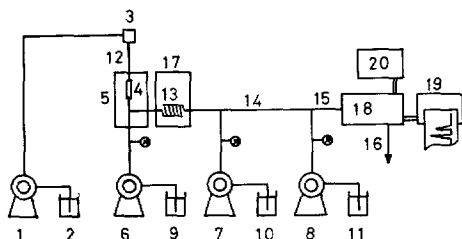


Fig. 1. Schematic diagram of the liquid chromatograph. 1 = Pump (1 ml/min); 2 = eluent; 3 = sample injector; 4 = analytical column; 5 = water-bath; 6–8 = acid-resistant pumps (0.4 ml/min); 9 = hypochlorite reagent; 10 = nitrite reagent; 11 = iodide reagent; 12 = PTFE tubing (0.5 m × 0.25 mm I.D.); 13 = PTFE reaction tubing (5 m × 0.5 mm I.D.); 14 and 15 = PTFE reaction tubing (0.5 m × 0.5 mm I.D.); 16 = PTFE suppressor tubing (5 m × 0.5 mm I.D.); 17 = water-bath; 18 = detector; 19 = recorder; 20 = data processor.

the post-column reagents, hypochlorite reagent (9), nitrite reagent (10) and iodide reagent (11), at a flow-rate of 0.4 ml/min each. Except for the tubing between 1 and 3 (stainless steel), the following PTFE tubing was used for all flows; transfer tube (12), the first reaction tube (13), the second reaction tube (14), the third reaction tube (15), suppressor tube (16). The temperature of the reaction tubing (13) was maintained at a suitable temperature by a water-bath (17).

The effluent from the post-column reaction was monitored at 370 nm with a Hitachi 635-T variable-wavelength detector (18) equipped with a flow cell (inner volume 8  $\mu$ l, path length 10 mm) in combination with a Hitachi 056 multi-range recorder (19) and a Shimadzu Chromatopac E1A data processor (20).

## RESULTS AND DISCUSSION

### *Separation of urea and related compounds*

To obtain a general idea of the separation process the cation exchange resin with water as the eluent, three different metal counter ions of the resin (lithium, sodium and potassium) were studied. These ionic forms of the resin and variations in the column temperature gave useful differences in both retention and selectivity. A representative chromatogram for the lithium-form resin is shown in Fig. 2. The capacity factors ( $k'$  values) determined are listed in Table I. Linear plots of  $\log k'$  versus  $1/T$  were obtained for each column (Fig. 3).

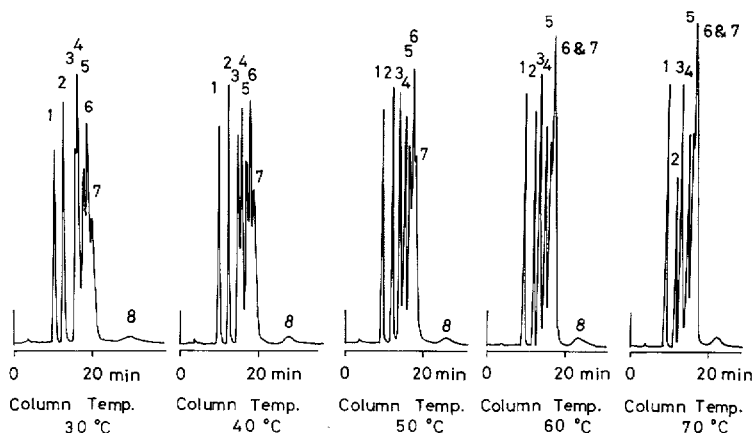


Fig. 2. Representative chromatograms using lithium-form resin. Chromatograms were obtained at the column temperatures shown. Values on the peaks represent the following compounds: 1 = allantoin; 2 = thiourea dioxide; 3 = thiourea; 4 = urea; 5 = N-methylurea; 6 = 1,1-dimethylurea; 7 = 1,3-dimethylurea; 8 = tetramethylurea.

The elution order of the substituted ureas is urea, N-methylurea, 1,1- and 1,3-dimethylurea and tetramethylurea, and is independent of the counter ion of the resin. These urea derivatives are neutral but are very polarizable molecules; the more polarizable compounds elute first and the interactions between the solutes and the resins involve hydrophobic interactions as a primary mechanism. The lithium-form resin exhibited larger  $k'$  values than the sodium and potassium forms. The water content of the lithium-form resin is higher than that of sodium- and potassium-form resins, and

TABLE I  
CAPACITY FACTORS ( $k'$ ) DETERMINED UNDER THE VARIOUS CONDITIONS

Compound	Resin											
	$\text{Li}^+$ form				$\text{Na}^+$ form				$\text{K}^+$ form			
	30°C	40°C	50°C	60°C	30°C	40°C	50°C	60°C	30°C	40°C	50°C	60°C
Allantoin	2.80	2.48	2.32	2.16	1.28	1.09	0.94	0.87	2.50	1.76	1.61	1.46
Thiourea dioxide	3.60	3.44	3.28	3.12	1.69	1.54	1.39	1.28	1.75	1.66	1.51	1.46
Thiourea	5.04	4.40	4.00	3.68	2.70	2.39	2.09	1.94	1.76	1.66	1.51	1.46
Urea	4.97	4.80	4.56	4.24	1.87	1.80	1.69	1.61	1.90	1.66	1.51	1.46
N-Methylurea	5.68	5.28	5.04	4.72	2.02	1.91	1.72	1.65	1.91	1.70	1.51	1.46
1,3-Dimethylurea	6.08	5.68	5.36	5.04	2.17	2.02	1.87	1.80	1.98	1.70	1.51	1.46
1,1-Dimethylurea	6.56	6.08	5.60	5.04	2.17	2.02	1.87	1.80	1.98	1.70	1.51	1.46
Tetramethylurea	10.2	9.28	8.56	7.76	3.83	3.57	3.29	2.83	3.43	2.76	2.54	2.24

polarizable molecules are readily partitioned into the hydrated resin phase of the lithium-form resin.

The selectivity of the low-molecular-weight urea derivatives depends to a large extent on the metal ion chosen. The lithium-form resin at 50°C is superior. On the sodium-form resin N-methylurea and 1,1- and 1,3-dimethylurea coelute and on the potassium-form resin no separation was obtained except for allantoin and tetramethylurea. Further, the elution order of highly polarizable molecules such as allantoin, thiourea dioxide, thiourea and urea varies with the metal ion of the resin. The selectivity can also be altered by varying the column temperature, as shown in Fig. 3. On the lithium-form resin, an addition of lithium nitrate to the mobile phase the urea/thiourea selectivity was reversed, as shown in Fig. 4, but the performance was not improved by the addition of salt to the mobile phase. In the proposed method, the lithium-form resin at 50°C was chosen as optimal.

#### Post-column detection of low-molecular-weight urea derivatives

In the proposed post-column reaction, it is assumed that both the rate of

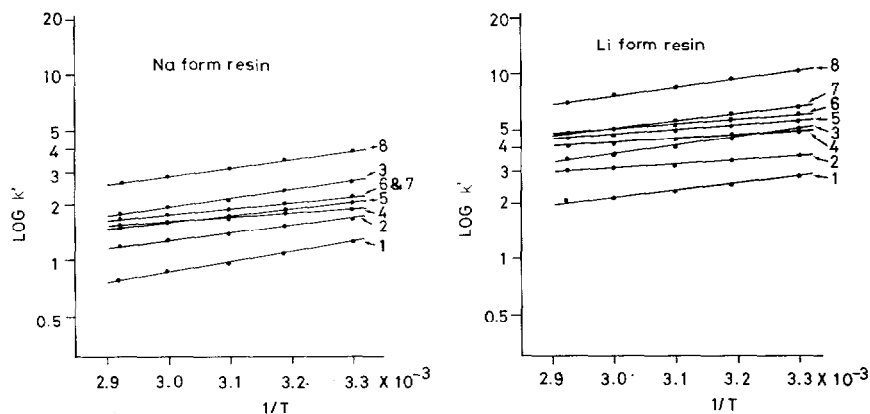


Fig. 3. Relationships between  $\log k'$  and the inverse of the absolute column temperature,  $1/T$ . Compounds as in Fig. 2.

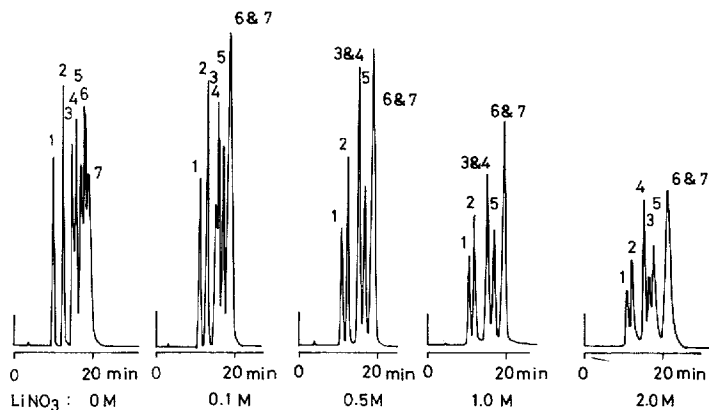


Fig. 4. Effect of salts in the mobile phase on the separation. Compounds as in Fig. 2.  $\text{LiNO}_3$  concentration as shown.

reaction and the species formed would vary with the pH of the reaction system and the reaction temperature. The influence of both the pH of the hypochlorite reagent and the reaction temperature on the sensitivity was therefore investigated.

*Effect of pH of hypochlorite reagent.* The sensitivity varied with the pH of hypochlorite reagent in different ways depending on the type of urea derivative (Figs. 5 and 6): allantoin; urea and substituted ureas; and thiourea derivatives. Allantoin had two sensitivity maxima at pH 5.0 and 7.5, that at pH 5.0 being the higher. For urea and substituted ureas, the sensitivity decreased with increase in pH of the hypochlorite reagent at both 35° and 70°C. Thiourea and thiourea dioxide showed different behaviours at reaction temperatures of 35 and 70°C. At 35°C, the sensitivities were fairly constant in the pH range 5–8 and below pH 5.0 the peak areas increased rapidly. At 70°C, the sensitivities had a minimum at pH 5–5.5.

*Effect of reaction temperature.* Figs. 5 and 7 show the effect of reaction temperature. The sensitivity of the determination of allantoin decreased with increase in reaction temperature at pH 5.0 of the hypochlorite reagent. For urea, substituted

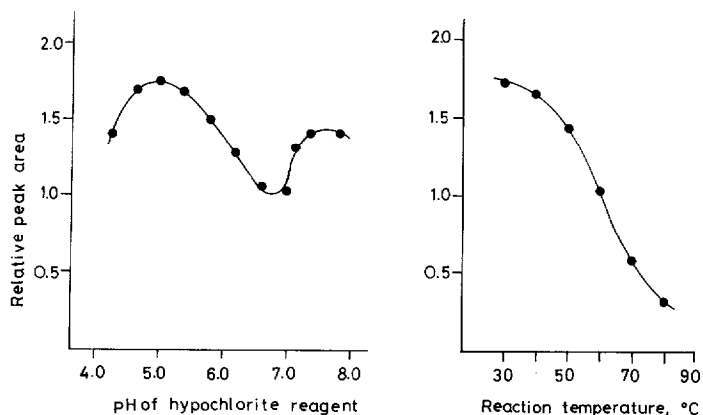


Fig. 5. Effects of (a) pH of hypochlorite reagent (reaction temperature 35°C) and (b) temperature of the reaction system (pH 5.0) on the sensitivity for allantoin.

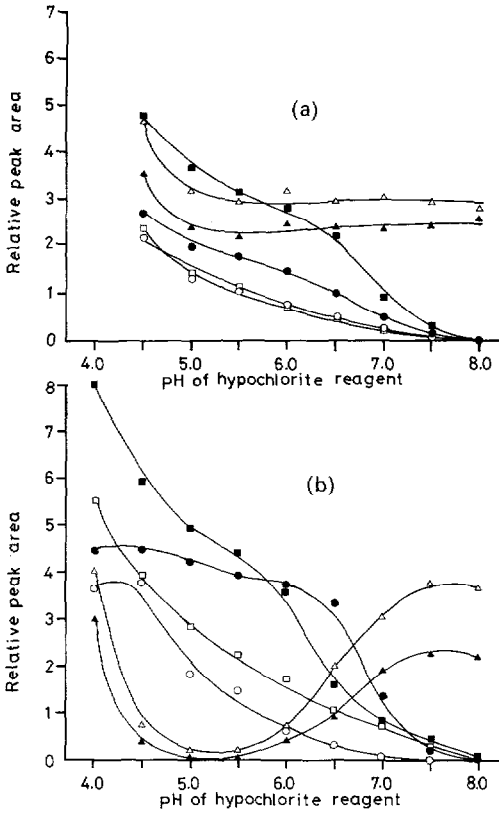


Fig. 6. Effect of pH of hypochlorite on sensitivity for urea derivatives. Reaction temperature: (a) 35°C; (b) 70°C. ○ = Urea; ● = N-methylurea; □ = 1,1-dimethylurea; ■ = 1,3-dimethylurea; △ = thiourea; ▲ = thiourea dioxide; ▼ = tetramethylurea.

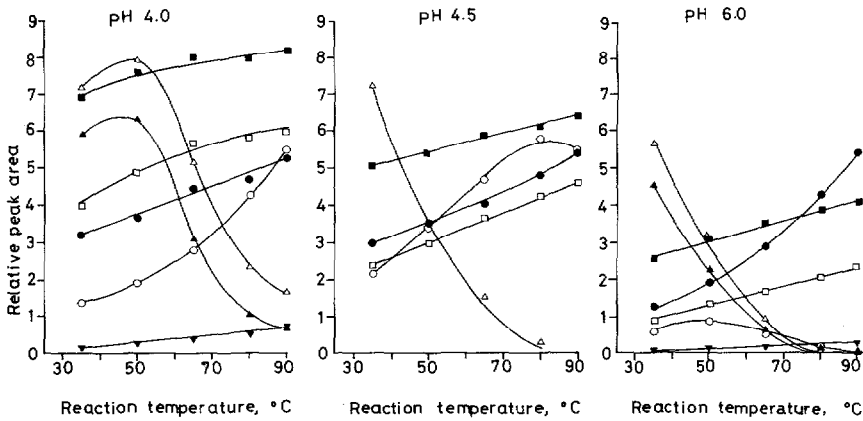


Fig. 7. Effect of reaction temperature on sensitivity for urea derivatives at different pH of the hypochlorite reagent.

TABLE II  
OPTIMUM POST-COLUMN CONDITIONS FOR UREA DERIVATIVES

<i>Compound</i>	<i>pH of hypochlorite reagent</i>	<i>Reaction temperature (°C)</i>
Allantoin	5.0	35
Urea	4.5	80*
Substituted ureas	4.0	80*
Thiourea derivatives	4.0	50

\* Above 80°C, the baseline became worse.

urea and thiourea derivatives, the effect of reaction temperature varied in different ways with the pH of the reaction system. With increase in reaction temperature, the sensitivity for urea increased at pH 4.0 of the hypochlorite reagent, but at higher pH (4.5, 6.0) the sensitivity had maxima at lower reaction temperatures. The sensitivities for substituted ureas increased with increase in reaction temperature, and the rate of increase gradually increased at higher pH of the hypochlorite reagent. The sensitivities for thiourea derivatives had a maximum at 50°C and pH 4.0 of the hypochlorite reagent. However, at higher pH of the hypochlorite reagent, the sensitivities decreased with increase in reaction temperature. The optimum conditions for each urea derivative are listed in Table II.

From these results, a pH of the hypochlorite reagent of 4.5 and a reaction temperature of 50°C were selected for the qualitative and quantitative analysis of urea derivatives. The calibration graphs were linear for each urea derivative. The detection limit of each urea derivative is less than 0.2 µg.

#### *Determination of low-molecular-weight urea derivatives in commercial products*

The accuracy of the method was tested by adding known amounts of urea, allantoin and thiourea dioxide to commercial standard products containing no urea derivatives (Table III). Quantitative recoveries were obtained, indicating that the proposed method can be employed for the determination of these compounds with wide applicability.

TABLE III  
DETERMINATION OF UREA DERIVATIVES IN STANDARD COMMERCIAL PRODUCTS

<i>Sample</i>	<i>Compound</i>	<i>Present (%)</i>	<i>Found (%)</i>	<i>Recovery (%)</i>
Shampoo	Urea	0.500	0.510	102.0
	Urea	5.00	5.08	102.0
Washing-up liquid	Urea	0.500	0.501	100.0
	Urea	5.00	4.96	99.2
Skin cream	Urea	5.00	4.98	99.6
	Allantoin	2.00	1.97	98.5
Drug	Allantoin	2.00	2.03	101.5
Reductive bleacher	Thiourea dioxide	5.00	5.06	101.2
	Thiourea dioxide	10.0	10.2	102.0

## REFERENCES

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