Table I shows that L_R decreases with gel compression, which indicates a more efficient separation. In addition, one can use L_R to calculate the minimum length of column required to separate two peaks, using data from an experiment in which incomplete or too great a separation occurs.

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Thin-layer chromatography of adenine and its metabolites

When large quantities of adenine are administered to various mammalian species, a portion of it is oxidized by xanthine oxidase to 2,8-dihydroxyadenine (DHA) which is extensively deposited in the kidney[1,2]. 2-Hydroxyadenine (2HA) and 8-hydroxyadenine (8HA) have been shown to be the minor and major intermediate, respectively, in this metabolic pathway[3]. In this communication, a thin-layer chromatographic (TLC) procedure for their separation and identification is described.

Materials and methods

Adenine (Kay Fries) and DHA (Aldrich) were commercial products. Adenine was further purified by recrystallization from water. 2HA (isoguanine) was prepared from isoguanine sulfate (Sigma). 8HA was synthesized by the reaction of phosgene on 4,5,6-triamino pyrimidine[4]. Solutions of approximately 1 mg/ml were prepared. DHA and 2HA were dissolved in boiling mixture of morpholine–water (50:50) while adenine and 8HA were dissolved in dimethylsulfoxide. Solutions were prepared fresh prior to use. Samples were applied with 3 μl capillaries* (Microcaps, Drummond Scientific Co., Broomall, Pa.) and solvent evaporated by using a hot air gun.

TLC was carried out on precoated Avicel, microcrystalline Cellulose powder F glass plates with fluorescent indicator (Merck, Darmstadt, G.F.R.) using the following solvent systems: (1) *n*-butanol–methanol–water–conc. ammonia (60:20:20:1); (2) isopropanol–water–conc. ammonia (70:30:1); (3) *n*-butanol–morpholine–diethylene glycol (MCB)–water (45:15:10:30). All solvents used were reagent grade. Spots were located using a short wave UV lamp.

* Use of microsyringes with metallic parts should be avoided as additional spots are observed due to degradation.

TABLE I

R_F VALUES OF ADENINE AND ITS METABOLITES

Substance	*R_F values in solvent system*		
	S1	*S2*	*S3*
Adenine	0.39	0.53	0.54
DHA	0.06	0.18	0.24
2HA	0.13	0.27	0.36
8HA	0.29	0.44	0.47

Results

Adenine and its metabolites have been separated in all three solvent systems used. R_F values reported are average of triplicate determinations (Table I). Applications of this method in determining the stability of these compounds in alkaline medium and their identification and determination in biological materials is in progress.

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