Organic Solvent Chromatography of Radioactive Sodium Pertechnetate

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We investigated the degree of chemical separation of radioactive sodium pertechnetate ($Na^+ TcO_4^-$) with various organic solvents. Chromatography results using nine different solvents were obtained and studied. Information, including temperature, humidity, pH, and background radiation, was recorded for the 135 chromatographic strips run during the study. We conclude that methylethylketone gives the best chemical separation and we recommend it as the organic solvent of choice when the presence of radioactive sodium pertechnetate is to be determined. The pH and temperature had no noticeable effect on results; however, humidity appeared to have a slight effect on the quantity of sodium pertechnetate that migrated up the chromatography strip.

We undertook a study to determine which solvent gives optimum separation of radioactive sodium pertechnetate on instant thin layer chromatography (ITLC) paper and to determine if such external conditions as temperature and humidity significantly affect chromatography results.

Materials and Methods

Radioactive sodium pertechnetate was eluted in saline 0.9% to yield stock solutions of approximately 75 mCi/ml. The samples used for testing were less than 0.1 ml. Approximately 1 μl of solution was actually spotted on the ITLC paper, which was 6.5 cm long and 0.75 cm wide. The solutions were allowed to run to the top of the chromatography strip. Most of the solvents migrated to the top in less than 1 min except butanol and butanol-containing solvents. The nine solvents used were methylethylketone (MEK), trichloroethane, acetone:chloroform 2:1, chloroform, acetone, butanol, ethanol, chloroform:glacial acetic acid:water 4:4:1, and butanol:ethanol:water 4:2:1. The vials used for developing the paper were 6.5 cm tall and filled with 0.5 cc of each solvent, which made the solution approximately 1.5 mm deep. The papers were spotted at the 0.5-cm mark. The cut paper method of reading chromatography paper was used; this method requires that the paper be cut in half after developing and each half counted in a well scintillation counter for 6 sec. The figures acquired are used to determine the percentage of sodium pertechnetate in each half. The optimum solvent would be the one that carried the majority of the sodium pertechnetate up the strip, leaving very little at the origin.

A pH paper with a range of 1–14 was used to test the pH of the daily elutions of sodium pertechnetate. Humidity was determined as percent relative humidity by the use of a wet bulb thermometer.

Results

From Sept. 1, 1981, through Sept. 14, 1981, a total of 135 chromatography strips were run. Preliminary data indicated that butanol and butanol-containing solvents developed slowly. Trichloroethane and ethanol were the only two solvents that did *not* consistently carry sodium pertechnetate up the chromatography paper; in fact, they gave good results only sporadically. All of the other solvents used in this experiment seemed to carry at least 95% of the sodium pertechnetate up the strip with MEK being consistently outstanding at 99% or better. Variations in the different pH readings of the sodium pertechnetate solutions did not appear to significantly affect the outcome of the chromatography (Tables 1 and 2).

Of the seven solvents that readily carried sodium pertechnetate, only MEK proved to carry more sodium pertechnetate than the others. Butanol and butanol-containing solvents were characterized by the slow rate at which the solution migrated up the chromatography strip—from 5 to 10 min. All other solvents migrated in less than 1 min.

Humidity did not seem to have a significant effect on the results of chromatography performed on MEK. The MEK results have been separated into two groups—those developed at humidity greater than 55% and those developed at humidity less than 55% (Table 3). The difference between these two groups was not statistically significant ($p = 0.1212$ student's t test).

Discussion

Our major finding was that MEK gives maximum separation of radioactive sodium pertechnetate. Many of the other solvents we used produced comparable values, but did not consistently produce this high de-

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TABLE 1. Chromatography Separation (Counts/0.1 Min) with Solvent, pH, and Humidity Variations

	Methyl-ethylketone	Trichloro-ethane	Acetone: Chloroform 2:1	Chloroform	Acetone	Butanol	Ethanol	Chloroform: Glacial Acetic Acid : Water 4:4:1	Butanol: Ethanol: Water 4:2:1	pH	% Relative Humidity
O	48	119837	68	119112	219	880	396	40	217	4.5	40
SF	50280	375	92371	58	109655	123105	117722	107723	112296		
O	323	116854	157	4	136	879	34426	138	1071	5.0	38
SF	83142	2	84642	118154	107078	115620	79093	116840	74370		
O	95	9553	4635	511	3522	878	34921	2081	1541	5.5	45
SF	115521	16104	81391	42673	70967	33135	899	41083	45145		
O	43	12756	179	543	5006	3086	111873	6431	4994	5.0	49
SF	120756	43476	53639	54312	98057	95083	3526	81492	106293		
O	16	2042	110	72	1459	1988	90474	16803	4933	5.0	61
SF	2833	108	106060	99231	106454	116252	1414	104234	118992		
O	45	70783	3177	304	15000	6076	120061	675	11826	4.5	30
SF	97526	766	70538	90143	71012	80135	250	4890	116111		
O	412	52290	279	216	528	594	899	898	433	4.5	75
SF	99024	691	2124	5274	8216	7131	39483	24382	4471		
O	315	49849	281	234	366	293	160	238	508	7.0	59
SF	75269	9849	40157	17818	11346	10235	86444	39483	26541		
O	46	70783	206496	198	3382	2226	554	2460	3773	5.0	25
SF	15031	762	53107	78670	69171	57980	87934	41116	5187		
O	428	32290	4191	98	1566	1461	2196	1652	689	4.5	91
SF	16385	16715	25644	31393	36582	43249	33962	41729	33961		
O	116	692	432	110	393	424	3186	8437	1959	4.5	33
SF	51721	81417	59249	51597	66646	96159	116377	68907	73739		
O	126	281	2900	2910	540	498	115	250	5540	4.5	64
SF	15764	40157	18990	99631	76203	92185	123136	120061	87934		
O	675	20614	657	115	3186	249	2910	352	160	4.5	59
SF	120425	53167	120425	123136	116317	128720	99631	125301	86445		
O	296995	290	542	120	72	391	110	948	412	4.5	60
SF	68990	68990	119076	79076	99024	76421	51579	121456	90260		
O	421	5261	6281	17641	67999	904	31393	62	962	5.0	71
SF	99631	42673	120120	120001	120761	88086	27854	39064	77075		

O = origin.
SF = solvent front.

gree of separation. The percent of humidity seems to affect the chromatography results, since ITLC paper can become partially humidified and loses its capability to draw solution up the strip. In addition, if ITLC paper gets wet or moisturized, this can affect the chromatography results for drugs that can become hydrolyzed. Hence, humidity probably affects ITLC paper only, not sodium pertechnetate. Therefore, we believe humidity can hinder chromatography results. The humidity we measured was that inside our hospital, which has an air conditioning system designed to keep humidity at approximately 55%. One can see that this varied from day to day, ranging from 25–91% (Table 1). Outside the hospital, the relative humidity averages 70% in New Orleans during the summer. The humidity also varies during the course of the day with the highest reading usually in the morning. Obviously, geographic location and time of day can affect the outcome of chromatography. Methylethylketone should be used in areas of high humidity since it is a less hygroscopic solvent (*1*).

The cut paper technique used in this study probably had a great effect on results. This method is frequently unreliable because many factors are assumed in using it. Where the paper is cut plays an important role; to be

TABLE 2. Chromatography Data Averages

Solvent	% Traveled up the strip
Methylethylketone	99.4
Trichloroethane	48.7
Acetone:chloroform 2:1	97.3
Chloroform	98.3
Acetone	96.6
Butanol	97.6
Ethanol	66.8
Chloroform:glacial acetic acid:water 4:4:1	95.7
Butanol:ethanol:water 4:2:1	95.9

TABLE 3. Comparison of MEK Results with Varying Humidity

Chromatography results of MEK on days with humidity < 55%	Chromatography results of MEK on days with humidity > 55%
99.9%	99.3%
99.9%	99.5%
99.9%	99.5%
99.9%	99.2%
99.6%	99.4%
99.6%	99.5%
99.7%	99.5%
	99.5%
Average 99.8%	99.4%

(A total of 15 strips were run.)

consistent, the chromatography paper should be cut in exactly the same spot each time. Scissors may become contaminated and hinder the results of all of the stips cut. The use of a chromatography scanner instead would be ideal.

In the hospital setting, time is an important factor. This could restrict the use of butanol and butanol-containing solvents.

Conclusions

Methylethylketone has proved to consistently carry the greatest percentage of radioactive sodium pertechnetate up the chromatography strip and we recommend it as the organic solvent of choice for this study. The eight other solvents tested could be used but would not reflect the closest estimation of the percentage carried up the strip. In a clinical situation, the small difference in results offered by MEK is probably insignificant. However, for a more accurate result in a questionable chromatography strip, MEK would be indicated. To help deter the effects of humidity on chromatography paper, we also recommend that a dessicant be placed in the container in which ITLC paper is stored.

The acceptable pH for a sodium pertechnetate elution ranges from 4.5–7.5. As long as an elution is in this range, no effect on chromatography will be observed.

References

1. Levit N. Addition to article on effects of humidity on TLC. *The Monthly Scan* October 1981.