## *Chromatography and Marathon Running*

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*The similarity of liquid chromatography (LC) and marathon running was investigated initially for the* Current Separations *humor issue. There are many parallels between the two, for example: column length, internal diameter and an ambient temperature affect on the arrival of analytes at the end of the column (retention time, and analytes with similar structures arrive close together). In a marathon, distance, road width and ambient temperature all affect the runners' race and finishing times, and athletes with similar skills finish close together. The parallels between sample preparation (such as solid-phase extraction) and warming up; column switching and picking up slow runners; and mobile phase composition and athletes' nutrition were considered. A key both to enjoyable running and good LC is to apply plenty of effort and ingenuity every day to achieve one's goals.*

It has been more than 30 years since I began to run on an embankment along the Tama River. At first, I could run only a few kilometers and would not have believed I would ever finish an entire marathon of 42.195 km, not once, but more than 100 times, and with a best time of 3 hours, 28 minutes and 24 seconds.

During the same time period I became an accomplished marathon runner, I developed simple and rapid analytical methods for amino acids in human plasma (1,2) and vitamins in foods (3-7) by liquid chromatography (LC) after sample preparation such as solid phase extraction (SPE) (8,9).

My experience suggests parallels between these two very different types of run. Runners and analytes both separate out, and this is affected by run parameters. Runners must maintain peak condition and to do so, they require proper nutrition and warming up. Samples must be prepared properly by SPE. Current LC analysis gives athletes vital information regarding the best intake of nutrients from food and supplements such as amino acids, vitamins and minerals, because measurements can be taken from human blood before, during and after a marathon run.

In this paper we deal with the relationship between two runs. We consider: separation of analytes or runners on the column or road; dead volume and distance from start to finish; sample preparation and warming up; column-switching and bus pick-up system for the late runners; maintenance and the athlete's daily regimen; and finally, chromatography and the determination to finish the race. Those comparisons are shown in **T1**. Last, I would like you to consider what you learn from comparing a marathon to running samples on a column.

## *The Experiment*

### Reagents and Materials

Chemical reagents were all of HPLC or analytical grade. Commercially available food samples and brown volumetric glassware were used.

All running gear, including shirts, pants, cap, gloves, socks, sunshades and shoes were available commercially. The watch, sensor chips, food and drinks were all officially approved.

### System Care

In LC analysis, equipment maintenance is vital to ensure good performance. Excessive retention on the column requires column-switching.

Runners are careful to avoid injury and bad habits, as athletes in poor condition either are unable to race or cannot perform well enough to finish the race. Those runners who fail to reach designated markers within specified time periods (e.g. 30 km within three hours), are picked up by bus and not allowed to finish.

### Apparatus and Conditions

The LC separation was carried out and samples were injected using Rheodyne Model 7125 manual sample loop injection valve.

The marathon runners, pumped up with enthusiasm, spread out over the 2001 Boston Marathon in a column 42.195 km x 3 m wide. An official model watch recorded arrival of runners and the 2001 official Boston bus acted as a switching device. About 20,000 runners started on the course. The fastest runners achieved a speed of 3 min/km at ambient temperature.

## *Results and Discussion*

### Separation

First, let us consider the separation of analytes and runners. In LC of food and biological sample constituents, separations are affected by column length, diameter, temperature and mobile phase composition. The mobile phase (nutrients) will contain important compounds such as methanol, acetonitrile, phosphates and ion-pair reagents. These compounds in the mobile phase are important to separate the analytes from co-existing compounds in complex samples. Flow rate (e.g., 1 ml/min) must be constant. In such controlled conditions, similar analytes elute close together on the chromatogram.

Marathon runners' performances are affected by, among other variables, length of the run, width of the road and ambient temperature. Their nutritional intake of amino acids, sugars, lipids, vitamins and minerals is crucial, both before and during the run. Many types of compounds, such as amino acids, vitamins and minerals, in a runner's blood play an important role during and after running in separating the runners from one another during the race and in recovery from muscle fatigue. We measure the compounds in a runner's blood by means of LC after clean up.

A constant running speed (e.g., 5 min/km) is advantageous. Under such conditions, runners of similar performance level finish close together. Second, we can look at dead volume and the starting line.

### Dead Volume

LC columns have a dead volume, which is important in evaluating the column.

A similar phenomenon is seen when starting a marathon. Only 30 to 50

runners can begin at the starting gun (no lost time; $V_1$=0), while many runners may wait 7 to 10 min to get going. Recent races at Boston, London, Hamburg, Paris, Vienna, Rotterdam and Katsuta have solved this problem by having a sensor chip inserted in the runners' shoes. This sensor adjusts the runners' starting time to when they actually cross the starting line.

An injected sample is crowded with many kinds of compounds that then separate over the length of the column according to their structures.

As a marathon begins, runners of varying stature are bunched together. During the run they separate in accordance with their abilities.

### Setting and Maintenance

Third, there are physical and maintenance effects (10). It is essential to prevent exposure of LC to direct sunlight, strong wind, dust, exhaust or acid fumes, and the supporting bench must be smooth and level.

Marathon runners, too, must be protected from direct sunlight, strong wind, dust and vehicle exhaust. The surfaces on which they run must be smooth and level.

In LC quality control, the key to good operation is consistent conditions, carefully maintained on a day-to-day basis. To run well, marathon athletes need a carefully balanced daily health regimen.

### Sample Preparation

Fourth, sample preparation and athletes' warm-ups can be compared.

A number of techniques have been developed to extend the application of LC, including pre- and post-column derivatization (12), column switching and SPE. A simple and rapid preparation method is very desirable for analyzing trace amounts of analytes in foods and biological fluids. Sample preparation is essential to ensure that subsequent LC analysis is effective. SPE can be compared with preparation for running.

To run a marathon successfully, a good warm-up using a well-designed technique is essential. Great effort and ingenuity are necessary to obtain good analytical data from LC; the hard work of sample preparation and clean-up makes it possible to enjoy the rest.

K. Yamada, winner of the 57th Boston Marathon in 1953, has said that hard work in training makes running the marathon enjoyable. Determination results in success, and reaching one target makes the next one clear (11). The key to obtaining good analytical results is choosing a suitable sample preparation method.

### Derivatization

Next, we can compare derivatization with interval running; both techniques help to separate faster runners from slower ones.

Pre- and post-column derivatizing reagents (12) have been developed for highly selective and sensitive detection of analytes and to improve their chromatographic separation from other sample matrix components. (The latter is also improved by using ion-pairing agents.) The best runners often use interval running as a tactic to separate themselves from slower runners.

### Internal standard

Having some internal marker is an advantage during running, both for samples and for marathon runners. Thus, internal standards and pacemakers can be compared.

Using an internal standard often can help to optimize an LC method. Such a standard should be structurally similar to the analytes but at the same time be well separated from them by the end of the run. Using a pacemaker alongside the marathon runners improves their speed. If several pacemakers were to run with the field, every runner's time might well be improved.

### Column-switching Technique

It is important that run times are kept within a reasonable limit. LC and marathons each have their own technique for this. Column-switching is a simple and rapid way of treating sample matrices, such as in analyzing drugs and their metabolites in biological fluids (13, 14) so convenient run times can be established. In a marathon, runners who fail to reach the end of a particular stage by a given time (e.g., 30 km within 3 h) are picked up by bus to clear the field.

### Chromatography

Next, consider chromatography itself in both LC and marathon runs where the runners show varying speeds over the course.

Other factors aside, when using ODS-type columns large molecular weight analytes show later running and more broadly spaced peaks than do smaller ones. Apparently, smaller, lighter analytes experience less drag on the column than the larger, heavier ones.

Although the distance is the same for all marathon runners, early finishers feel better and experience less muscle fatigue. These faster runners tend to be leaner. It has been calculated that each footfall produces a load of about three times body weight on a level course, and four times if the course is downhill. Thus, the lighter runner is understandably less fatigued than the heavier one at the end of the race.

The presence of compounds such as acetonitrile in the mobile phase aids analysis by LC by producing sharper peaks. In a marathon, those runners who are well-nourished show improved performance and converge in the upper ranks of competitors.

### Determination

Last, analytical data and finishing time show that run times can be better determined for consistent runners in both LC and in marathons.

In LC, the peak height ratio of analyte and internal standard is plotted against the amount of analyte. Satisfactory linearity is shown over the range of 0.1 to 10 ng on column (e.g. Y = 0.342 X - 0.023, Y = peak:height ratio, X = amount of analyte in ng). We can estimate the amount of analyte from the calibration graph. Higher recoveries of analyte and smaller relative standard deviation (RSD) are preferred.

In a marathon, the faster runners show such a constant pace when measured lap by lap that their finishing times can be calculated from their average lap time. Satisfactory linearity is also shown over the range of 1 to 42.195 km (e.g. Y = aX, Y = finishing time, a = lap time/km [e.g., 3 min/km], X = distance [km]). Olympic-class runners have very similar finishing times (smaller RSD on each marathon) and show the greatest consistency during the race. Analytical data and finishing times demonstrate this.

## *Conclusion*

This paper has examined the parallel features of LC and marathon running. Separation, dead volume, sample

preparation, column-switching, derivatization, chromatography and calibration graphs are all considered along with their parallels.

I would like to pose two questions. ***A.*** What is achieved by running marathons, or samples on LC? ***B.*** What do the participants get out of coming to the top of the column or to the starting line? A top runner puts great effort into training and shows a great deal of diligence in improving on his or her record, even if only by a few seconds. Breaking such a record has little immediate effect on everyday life, but the efforts and ingenuity that have been applied eventually bring great enrichment. For example, pharmaceutical research, development of food supplements and improving the quality of running shoes and clothing will lead to better medicines, foods and clothing. The advance of technology serves the needs of the runner, just as the advance of science pushes toward better health.

I ask the question: What is the experience of the runners, analytes at the end of the column and athletes at the finish line? Applying substantial effort and ingenuity every day is key to realizing one's dreams, whether they are of enjoyable running or good LC.


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**T1.** Similarity of liquid chromatography and a marathon.

| Liquid Chromatography | Marathon |
|---|---|
| *Sample* | *Many runners* |
| Analyte | Runner |
| Co-existing compounds | Fellow runners |
| *Apparatus and Conditions* | |
| Conditioning time | Warming up |
| Injection | Starting line |
| Analytical column length | Distance |
| Column diameter | Road width |
| Column temperature | Ambient temperature |
| Dead volume | From here to starting line |
| Detector | Finish line (goal) |
| Mobile phase | Runner's blood |
| (e.g. methanol, acetonitrile) | (e.g. nutrients, amino acids, vitamins, minerals) |
| Stainless tube | Runner's artery (vein) |
| Flow rate | Running speed |
| HPLC pump | Runner's heart |
| Reagents and materials | Running shoes, clothes, watch, aid station |
| Sample preparation | Daily training |
| SPE | Pre-running before marathon |
| Labeling | Interval running |